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Changes in red cell density and related indices in response to distance running

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Summary. The red cell population in peripheral venous blood was characterised in 7 young males before and up to 16 days after a 21.1 km road race.

There was a $1.9 \pm 2.4\%$ (mean $\pm$ SD) reduction in plasma volume immediately post race ($p < 0.05$), an increase in serum osmolality from 277 ± 4 mOsm·kg^{-1} to 291 ± 14 mOsm·kg^{-1} ($p < 0.05$) and a reduction in red cell water ($64.4 \pm 0.3\%$ to $63.4 \pm 0.4\%$, $p < 0.001$). The latter was consistent with alterations in the manually derived MCV and MCHC values although the same Coulter derived values were unaltered. A concomitant increase in median red cell density in whole blood (1.1045 ± 0.0009 g·ml^{-1} pre race to 1.1057 ± 0.012 g·ml^{-1} immediate post race, $p < 0.05$) was recorded by centrifugation through phthalate esters of different density. The changes in creatine content of the red cells suggested that during the race younger cells were released into the circulation but that 24 h to 72 h after the race the mean red cell age had increased. Similarly, fractionation of the red cells on discontinuous Percoll density gradients indicated that the cell population was significantly denser in all post race samples up to 72 h but had normalized by a 16 day sample; the osmotic fragility was similarly affected. The reticulocyte count did not significantly increase throughout the experiment but the mean red cell creatine content was elevated 16 days post exercise compared with the pre-race value suggesting a possible increase in erythropoiesis between 72 h and 16 days post race. The study shows that exercise of this type may induce a small reduction in mean red cell survival time but that this does not immediately change erythropoietic activity.

Key words: Erythrocyte aging — Erythrocyte indices — Exercise — Exertion

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Introduction

Certain forms of physical activity such as distance running may not only cause direct red blood cell damage and intravascular haemolysis (Davidson 1969; Platt et al. 1981) but also accelerate red cell aging and impair deformability (Reinhart et al. 1983; Galea and Davidson 1985). Although red cell survival has been most effectively studied in pathological states by the use of radiolabelled cells (Freireich et al. 1957) current ethical standards virtually and rightly preclude the application of such techniques for research purposes in healthy subjects. In such circumstances an alternative and sufficiently sensitive method of studying red cell survival is that of red cell distribution in a density gradient, as cell density has been correlated with cell age (Danon and Marikovsky 1964; Piomelli et al. 1967) although the nature of the relationship has been questioned (Clark and Shohet 1985; Janson et al. 1986).

This study involving competitive runners attempts to establish evidence of reduced red cell survival by determining several age-related variables and to correlate the alterations in indices with changes in body fluids and their distribution. The work reports not only the changes detected immediately after a 21.1 km road race but also their serial behaviour over the subsequent 16 days.

Methods

Haematological parameters were investigated in 7 male subjects, age 26±4 years (mean±SD), height 180±6 cm, body weight 73.9±9.1 kg participating in a half marathon road race (21.1 km) run under favourable conditions (16—18°C, light winds) at the end of August 1986. Five of the subjects were recreationally active and 2 undertook light training. Nude body weight was recorded before and after the race to the nearest 100 g.

Blood sampling. Blood was withdrawn from an antecubital vein with the subject in the sitting position 2 days before and 1 h before the race, immediately (within 5 min) and 1, 2, 3 and 16 days after the race. Except on the day of the race, samples were taken between 0800 and 0900 h after overnight fasting. No dietary restrictions were imposed on the day of the race. Subjects did not train between the end of the race and the taking of the sample on the third day but thereafter resumed normal activity. The blood (8 ml) was dispensed into 2×4 ml containers with K_2EDTA as anticoagulant and a separate aliquot (1 ml) allowed to clot for serum osmolality measurement.

Haematological analysis. Peripheral blood profiling (Coulter S Plus IV) has been described previously (Davidson et al. 1987); manual haematocrit values, PCV(m), were determined by the conventional micromethod using a Hawksley centrifuge and centrifugation at 12000g for 3 min; reticulocytes were counted on a film pre-stained with New methylene blue (Dacie and Lewis 1984) and expressed in absolute numbers ($\times 10^9 \cdot l^{-1}$).

The percentage changes in plasma volume PV were calculated from the values obtained for PCV(m) and haemoglobin (Hb) according to the formula of Dill and Costill (1974). Mean red cell volume (MCV) and mean cell Hb concentration (MCHC) were derived from both the Coulter profile, denoted by (c), and by calculation, denoted by (m), from the PCV(m) and Coulter red cell count (RBC): MCV=(PCV(m)×10)/RBC, MCHC=(Hb×100)/PCV (Stäubli and Roessler 1986).

Serum osmolality was measured by an osmometer (Wescor Inc, Utah, USA), dependent on reduction of vapour pressure. The cell water content expressed as a percentage of total weight was calculated from the difference in wet and dry weight of 1.00 ml of whole blood making use of the PCV(m) and correcting for plasma weight by drying 0.50 ml under the same conditions (43°C, 48 h).

Red cell osmotic fragility, measured in HEPES buffered saline (pH 7.4) over a range of 154 mM (0.90% w/v) to 17 mM (0.10% w/v) NaCl (Dacie and Lewis 1984), was expressed as concentration of NaCl causing 50% lysis i.e. median corpuscular fragility (MCF).

Red cell creatine was measured in a volume of packed cells of known PCV (70—80%) by the diacetyl-1-naphthol reaction (Li et al. 1982) and recorded both as $mg \cdot l^{-1}$ cells and fg/cell (lfg=10^{-15} g).

Cell density measurements. Red cell density was determined by two methods using, (a) whole blood and (b) isolated red cells resuspended in an artificial medium of constant osmolality (300 $mOsm \cdot kg^{-1}$ H_2O) and pH (7.4):

(a) Whole blood, after continuous mixing for 15 min, was layered onto phthalate esters at six different specific gravities (range 1.094—1.114 $g \cdot ml^{-1}$) contained in microhaematocrit tubes. After centrifugation (12000 g, 15 min), the density of ester permitting 50% of the cells to pass through was calculated for each blood sample and recorded as the median cell density (MCD, Danon and Marikovsky 1964).

(b) Following removal of white cells and platelets by an alpha-cellulose:micro-crystalline cellulose (1:1) filter (Beutler et al. 1976) the red cell population was fractionated on a Percoll (Pharmacia, UK) discontinuous density gradient (Rennie et al. 1979). The percentage recovery of Hb from the cellulose filter ranged from 88.6% to 96.4% with no difference between samples obtained at the different times. Cells were layered onto the prepared 8 ml gradient and centrifuged for 5 min at 1000 g. The cell fractions were separated by sequential aspiration of 1 ml between the mid points of each layer since the majority of cells separated at the layer interfaces. There was no loss of cellular Hb in the Percoll gradient. The cells were washed, resuspended in buffer, the Hb content of each fraction measured by the cyanmethaemoglobin method and the results expressed as 4 fractions.

Statistics. All data were analysed by ANOVA for repeated measures followed by the Duncan multiple range test or by Student's *t*-test for paired data. A probability (p) of less than 0.05 was considered adequate to reject the null hypothesis. For purposes of statistical analysis the immediate post race sample was compared to the immediate pre-race sample, and the 24 h, 48 h, 72 h and 16 day post race samples were compared to the 48 h pre race sample since these were all drawn from overnight-fasted subjects. The data were expressed as mean values ± standard deviation.

Results

The subjects (n=7) completed the race in 96.7±12.2 min and all showed a reduction in body weight (73.9±9.1 kg to 72.4±9.2 kg; $p<0.001$). The 'self assessed' fluid intake during the race was 410±400 ml.

Haematological changes associated with fluid shifts

During the race. There were significant increases in serum osmolality, Hb concentration and in PCV(m) (Table 1) with a resultant reduction (1.9±2.4%; $p<0.05$) in the calculated plasma volume. A similar reduction in percentage red cell water (1.5±0.5%, $p<0.001$) was also recorded. This loss of cell water was reflected by an accompanying increase in MCD ($p<0.05$) as measured by the phthalate ester method for whole blood.

The Coulter values of MCV(c), MCHC(c) and red cell distribution width (RDW) did not alter significantly. However the value of MCHC(m) calculated from the PCV(m) were significantly ($p<0.01$) increased from 339±3 to 350±7 $g \cdot l^{-1}$ over the race period. Similarly, MCV(m) differed from the MCV(c) and showed a reduction (2.4±2.2%, $p<0.05$) commensurate with the loss of cell water.

Post race changes. The haemoconcentration observed on completion of the race (Table 1) was

Table 1. Haematological changes (mean ± SD) associated with fluid shifts. For the purpose of statistical analysis the post race values between 24 h—16 days were compared to the 48 h pre race values and the immediate post race with the immediate pre race value; * $p<0.05$, ** $p<0.01$, *** $p<0.001$

	Pre-race		Post-race				
	48 h	1 h	<5 min	24 h	48 h	72 h	16 days
Hb ($g \cdot l^{-1}$)	148 ±6	144 ±5	150** ±4	141 ±7	138** ±4	138 ±6	145 ±9
PCV(m) ($l \cdot l^{-1}$)	0.43 ±0.01	0.42 ±0.02	0.43 ±0.01	0.41 ±0.02	0.41* ±0.01	0.40 ±0.01	0.43 ±0.03
Red cell water (%)	63.7 ±0.4	64.4 ±0.3	63.4*** ±0.4	64.5*** ±0.4	64.1 ±0.7	63.9 ±0.4	63.4 ±0.5
MCD ($g \cdot ml^{-1}$)	1.1045 ±0.0009	1.1045 ±0.0009	1.1057* ±0.0012	1.1026** ±0.0011	—	1.1042 ±0.0013	1.1054* ±0.0014
Serum osmolality (mOsm·kg)	284 ±3	276 ±4	291* ±13	278*** ±5	286 ±7	284 ±6	293* ±2
PV changes (% increase)	0	—	—	5.4* ±6.5	8.2** ±5.3	9.8*** ±3.9	−0.6 ±7.3

not only reversed but replaced by a significant ($p<0.01$) expansion of plasma volume at 48 h compared to 48 h pre race. The mean serum osmolality recorded 24 h after the race was lower than the 48 h pre-race value ($p<0.001$) and the percentage red cell water was increased ($p<0.001$) with a fall in MCD ($p<0.01$). The last three variables returned to their pre-race values within 72 h but the plasma volume remained significantly greater ($p<0.001$).

Serum osmolality was found to be elevated in all subjects at 16 days after the race ($p<0.05$) and MCD was increased ($p<0.05$) although red cell water was not altered.

Changes in age related haematological parameters

Although red cell distribution in a Percoll gradient showed some variation in the pre-race samples, following the run there was a shift in the cell population towards the higher densities in all subjects (Fig. 1). The changes were still evident ($p<0.05$) 72 h after the race but after 16 days the density profile, while still containing a significant proportion ($p<0.05$) of more dense cells, showed a return towards the pre-race distribution as there was no longer a significant difference in the lighter fraction. The median cell fragility (MCF) followed a similar pattern of behaviour (Table 2), except that the increase in MCF observed immediately after the race ($p<0.01$) was even more pronounced at 72 h ($p<0.001$). The MCV(m) values recorded ($p<0.05$) between 24 h and 72 h were all greater than that recorded in the 48 h pre-race samples, while the MCHC(m) values were reduced ($p<0.05$).

The red cell creatine content (expressed in fg per cell) increased over the period of the race ($p<0.05$, Table 2) but was significantly reduced 48 h post race ($p<0.05$). Sixteen days after the race the red cell creatine ($p<0.05$) was significantly elevated over the pre race values while the

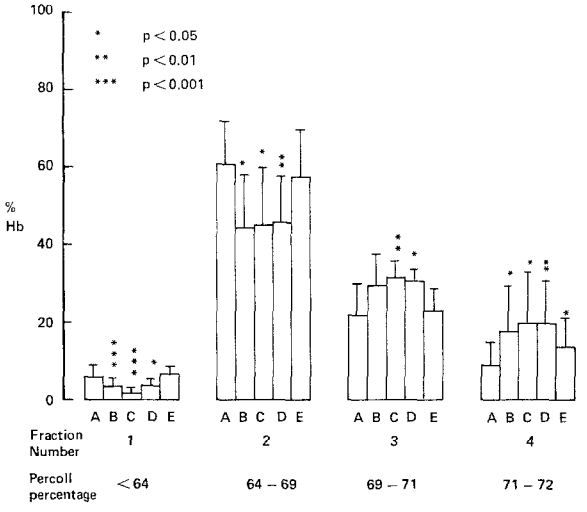

Fig. 1. Distribution of cellular Hb in a 4 fraction discontinuous Percoll density gradient. Each of the 5 columns for a given Percoll concentration represents a different sample time: A — 48 h pre-race; B — immediately post-race; C — 24 h, D — 72 h and E — 16 days post-race. Mean with SD bar are given. Statistical analysis was on paired values for each subject in each gradient fraction with the post race being compared with the pre race values. Symbols of significance are given in Tables 1 and 2

Table 2. Red cell age-related indices. Statistical analysis as in Table 1

	Pre-race		Post-race				
	48 h	1 h	<5 m	24 h	48 h	72 h	16 days
Cell creatine (fg/cell)	6.47 ±0.95	6.65 ±1.04	6.74* ±1.01	6.49 ±0.95	6.34* ±0.92	6.45 ±0.88	7.36* ±1.52
MCF (mM NaCl)	76.8 ±3.1	—	77.7 ±3.4	79.1** ±3.9	—	79.7*** ±3.1	77.0 ±2.7
MCV(m) (fl)	87.4 ±3.6	90.2 ±4.6	87.9* ±3.1	88.9* ±3.8	89.5* ±4.3	90.0** ±2.5	90.4* ±5.6
MCHC(m) ($g \cdot l^{-1}$)	348 ±5	339 ±3	350** ±7	340* ±4	339 ±4	340* ±9	340* ±5
Reticulocytes ($\times 10^9 \cdot l^{-1}$)	98 ±29	80 ±38	93 ±44	94 ±38	46** ±37	63 ±23	61 ±24*

number of reticulocytes was slightly reduced ($p < 0.05$, Table 2). The creatine content of cells ($mg \cdot g^{-1}$ Hb) separated by the Percoll gradient technique decreased with increasing cell density ($p < 0.001$, Table 3).

Over the experiment red cell creatine showed similar changes (Table 3) in distribution to Hb (Fig. 1) but, although there were fewer cells in the lightest fraction (1) up to 72 h following the race, these cells had a higher creatine concentration indicating that mean age was therefore younger. At 16 days the creatine content of the cells in fraction 1 was still elevated ($p < 0.05$), while the percentage of total creatine in the fraction had returned to pre race levels.

Discussion

Red cell fluid changes. Because of fluid loss, a period of severe physical exercise may result in a decrease in body weight, haemoconcentration and an increase in serum osmolality (Dill and Costill 1974; Maughan et al. 1985). In this study we further observed that the increase in serum osmolality was associated with an increase in red cell median density and that the increase in intracellular concentration of both haemoglobin and creatine and reduction in cell volume were consistent with the decrease in red cell water content. Our manually derived data not only support other studies demonstrating that distance running (Stäubli and Roessler 1986) and endurance training (Brodthagen et al. 1985) are associated with an immediate post-exercise reduction in MCV, but also serve to emphasise the importance of recognising the variations and limitations of the electronic methods of measuring red cell indices (Mohandas et al. 1980). In addition, although both MCV(m) and MCHC(m) have been shown to be age dependent variables (Tillman et al. 1980), fluid shifts make these unreliable measures of cell age in this present experiment.

Table 3. The distribution of red cell creatine (%) and intracellular creatine concentration ($mg \cdot g^{-1}$ Hb) in Percoll density gradients. The values are for fractions 1 and 4 as defined in Fig. 1 and statistical analysis as cited in Table 1. The column lettering from Fig. 1 is given below the sample times

		Pre-race		Post-race				
		48 h (A)	1 h (—)	<5 min (B)	24 h (C)	48 h (—)	72 h (D)	16 days (E)
Distribution of creatine (%)	Fraction 1	8.0 ±4.6	—	5.0 ±4.8	3.7** ±3.1	—	5.3** ±3.8	8.4 ±3.6
	Fraction 4	3.6 ±3.2	—	10.1 ±8.1	11.3** ±5.6	—	12.4** ±9.1	5.1 ±4.9
Creatine concentration ($mg \cdot g^{-1}$ Hb)	Fraction 1	0.22 ±0.06	—	0.24 ±0.09	0.26* ±0.08	—	0.25 ±0.10	0.25* ±0.08
	Fraction 4	0.07 ±0.06	—	0.09 ±0.04	0.10 ±0.03	—	0.09 ±0.04	0.06 ±0.06

Red cell age. The main objective of our study was to detect any change in mean cell age after prolonged running by monitoring red cell creatine and density distribution, commonly recognised age-related red cell indices (Fehr and Knob 1979; Piomelli et al. 1967). The measurement of cell density using whole blood and phthalate ester centrifugation is clearly dependent on the serum osmolality and indeed we used this method both to confirm the measurement of red cell water and establish the physiological red cell density changes. The density distribution of cells in the Percoll gradients is artificially dissociated from the osmolality changes since the cells were washed and resuspended in isotonic medium and therefore the pattern more truly reflects the differences in cell composition and shape maintenance that may occur between sample times. The distribution of red cells in such gradients has been correlated with age (Rennie et al. 1979) although the relationship is disputed (Clark and Shohet 1985; Janson et al. 1986). However we have also measured two other age related variables, osmotic fragility (Marks and Johnson 1958) and red cell creatine (Fehr and Knob 1979). The persistent changes in red cell density distribution between 24—72 h after the race, the increase in MCF and reduced red cell creatine all suggest that the red cell population was older. The density behaviour suggests that there is no differential destruction of older cells (Reinhart et al. 1983), since this would cause a shift to the lighter fractions. On the contrary, the behaviour can be explained by a maturation of circulating reticulocytes which are significantly reduced after the race, or aging of all circulating cells. Recent studies have shown alteration in red cell morphology following distance running (Reinhart and Chein 1985; Hales et al. 1986) but a study of red cell survival using radiolabelling techniques failed to demonstrate any significant changes in a group of well-trained female marathon runners (Steenkamp et al. 1986).

Interpretation of changes involving age-related determinants may be complicated by mobilisation of sequestered red cells from the splanchnic and splenic circulations. This latter effect is of importance in horses (Persson et al. 1973), but is thought to be of minor importance in man due to the smaller red cell splenic pool (Ebert and Stead 1941). Quantitatively, the red cell creatine increase (per cell) during the race can theoretically be accounted for by the release of approximately 7 ml (in a circulating volume of 5000 ml) of young cells which is double that estimated in a previous study (Keys and Taylor 1935) or removal of approximately 22 ml of old cells, nearly tenfold that calculated from the elevation of plasma haemoglobin released from lysed cells (Chaplin et al. 1961). However, the elevation of creatine immediately post race is not consistent with the density or MCF data.

Red cell formation. The alteration in cell density distribution and age did not appear to lead to an increase in erythropoietic activity between 24—72 h after the race, as assessed by the reticulocyte count and red cell creatine. However, the elevation of mean red cell creatine and the return of the population to lighter densities by the sixteenth day do suggest that additional younger cells may have subsequently been released into the circulating pool and/or older cells removed. This alteration in red cell age occurring by 16 days after running is in accordance with the higher ATP and 2,3-diphosphoglycerate levels recorded in trained athletes (Brodthagen et al. 1985) and reflecting overall the younger red cell population (Mairbäurl et al. 1983).

In conclusion, competitive distance running seems to cause an increase in red cell age and therefore a reduction in red cell survival time without an immediate erythropoietic response. Experimentally, red cell creatine content provides a sensitive measure of the overall changes in the cell population and from the distribution of cells in a density gradient it was possible to conclude that the aging process appeared to be general to cells of all ages. Because MCV and MCHC were greatly altered by the serum osmolality changes associated with the exercise they are therefore less reliable indicators of red cell age.

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