

Leucocyte and erythrocyte counts during a multi-stage cycling race ('The Milk Race')

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Venous blood samples were taken from eight competitors in mid-evening after a racing day, and in the early morning before the next day's race, three times during the course of the Milk Race, 1992. These were used to gather information about the changes in circulating leucocyte levels in response to the exceptionally high sustained daily workload required during a major multi-stage race. The primary objective was to provide knowledge of 'normal' values against which future clinical judgements of abnormality might be made in these unusual circumstances. During the race, estimated energy output was about 25 MJ (6000 kCal)/day. The mean total circulating leucocyte numbers (per litre of blood), and those of individual leucocyte classes (neutrophil, lymphocyte, monocyte, eosinophil and basophil) were all inside the normal range both in the morning and in the evening. Evening counts were, however, 30–50% higher than morning counts, for all classes except eosinophils. We conclude that individual clinical decisions about leucocyte levels can best be made using normal (sedentary man) values if a morning sample is taken.

Keywords: heavy exercise, leucocytosis, cycling, circadian

This brief study was initiated after reading a newspaper report that a competitor in the 1991 Tour de France had been advised to retire from competition because 'Doctors had found a high white cell count' and suspected a viral infection. No more information than this was given, but it raised the question in our minds 'What is *high* in the context of the Tour de France?' What standards of normality were applied? Exercise is well-known to induce a substantial leucocytosis, and this may be both transient and delayed, the latter being relatively persistent, even when induced by a limited period of severe exercise¹. We decided that it would be useful to seek normal values in the unusual context of a major cycle stage race, by sampling in the two periods when it seems most probable that a competitor might present himself to a doctor with

complaints of feeling unwell; namely at the end of a day (in the mid-evening), or early in the morning, soon after waking.

A delayed leucocytosis (at 3 h into recovery) has been reported in response to single bouts of exercise², or even to double bouts of exercise in the laboratory³ that is typically a pure neutrophilia, although following 3 h of exhaustive exercise, monocytosis and eosinopaenia were noted in recovery⁴. It has been hypothesized that the delayed peak in neutrophil leucocytes is induced by the earlier elevation in cortisol level caused by the exercise. It is well-known that cycle stage races involve massive workloads, which have been assessed to amount to a mean energy expenditure of about 25 MJ and a maximum of about 32.5 MJ/day (6000 and 7800 kCal/day respectively) during the Tour de France⁵. It is also known that cortisol turnover is normally related to energy turnover during exercise⁶. Such high levels of work are therefore bound to involve relatively high cortisol outputs, which might be expected to exert some influence on white cell numbers.

Methods

Eight highly trained young male competitors in the Great Britain and England teams participating in the Milk Race, 1992, gave their written informed consent for this study, which had the approval of the Tower Hamlets District Ethical Committee. They ranged in age from 23–27 years, mean(s.d.) weight was 69.3 kg(2.77), and mean(s.d.) height 1.78 m(0.06).

Venous blood samples were taken before and after (evening/morning) the night before the race, and over the fifth, eighth, and twelfth nights of the race, in each of these cases following a hard day of racing (of 115.5, 73.4, and 105.6 miles respectively). The evening samples were taken after the evening meal, at approximately 2030–2100 h, and the morning samples were taken soon after waking, at 0700–0900 h. The evening samples were taken 5–7 h after finishing racing for the day. Blood for cell counts was withdrawn (without stasis) from an antecubital vein into 5 ml Vacutainers containing Na-EDTA. Whole-blood tubes for cell counts were wrapped in polythene bags and floated on polystyrene chips

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above ice-water in a wide-mouthed 41 Dilvac flask for transport back to London soon after collection of the morning samples. With the unfortunate exception of the control samples taken on the night before the race (in which the white cell results were spoiled by excessive chilling and too long a delay in reaching the counter), they were counted later that day by an automatic cell counter, (Model H-1, Technicon Instruments, Basingstoke, UK). Values reported and analysed here included: counts of total leucocytes, neutrophils, lymphocytes, monocytes, eosinophils, basophils, and red cells (all white cells $\times 10^9/l$, red cells $\times 10^{12}/l$), haemoglobin concentration, g/dl; packed cell volume, % (PVC); and three red cell indices mean cell volume, fl, (MCV), mean cell haemoglobin, pg, (MCH) and mean cell haemoglobin concentration, g/dl, (MCHC).

The statistical package Instat (Statistical Services Centre, University of Reading) was used to analyse the results on an Archimedes 440/1 Computer (Acorn Computers Ltd). Seven of the subjects each contributed samples at all of the sampling times; the eighth subject fell heavily late in the race, and so withdrew before the last two samples were taken. In analysing the overnight changes in the cell counts during racing, there was no significant change in the individual results during the progress of the race. In order to avoid weighting effects that can be caused by missing values, the values for the three (or two) evening samples for each subject, and the values for the three (or two) morning samples for each subject, have been averaged, and it was these average values that were then used in testing for differences between morning and evening (using a paired Student's *t* test). Differences were accepted at the $P < 0.05$ level of significance.

Results

White cell counts

The total white cell count did not rise significantly with the progress of the race; the mean total white cell counts on six occasions are shown in Figure 1. The very slight tendency for the counts to rise later in the race was not statistically significant, and any slight individual differences might have been due to the difficulty of the particular day's stage, rather than to any underlying progressive effect of the cumulative stress of the event. For further analysis, therefore, the means of the cell counts in each of the eight subjects sampled in the evenings after racing, or on the

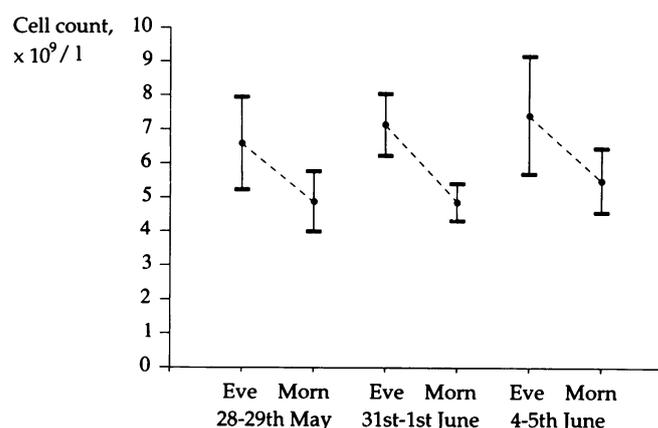


Figure 1. Leucocyte counts on samples taken before and after the fifth, eighth and twelfth nights of the race. The cumulative distance covered was 387.2, 655.3 and 946.7 miles (622.5, 1053.2, 1551.1 km) at these three points in the race. In calculating the 95% limits of the range about the mean for this figure, the appropriate value of statistic *t* was applied (in relation to the number of degrees of freedom upon which each sample s.e.m. has been based)

following mornings, were used for between-individual analysis as recorded in Table 1; paired *t* tests were then based upon analysing these means (i.e. a single pair of values represented the results on each subject). With only one exception, individual values recorded were 'normal', i.e. within the appropriate reference range for healthy adults⁷ (see Table 3), although the evening values were distinctly higher (usually 30–50%) than the morning values. The exception lay in the eosinophil counts, partly because in this case there was no change between evening and morning samples, and also because one individual had an eosinophil count in all samples that was consistently above the normal reference range for adult males (being from $0.59-0.72 \times 10^9/l$). The levels in this individual are thought to have been unrelated to his participation in the race, and his results for this variable have therefore been excluded from further analysis.

Haemoglobin and erythrocyte counts and associated indices

The red cell counts and the PCV in this group of extremely fit cyclists were inside the normal reference range, but were close to its lower end (Table 2). The MCV was normal, and slightly smaller in the evening

Table 1. White cell data, with overnight changes

	Evening	Morning	Differences	Differences as % morning
Leucocytes	6.96(1.92)	5.04(0.79)	1.92*(1.28)	48.0
Neutrophils	3.74(0.98)	2.52(0.56)	1.22*(0.72)	48.4
Lymphocytes	2.31(0.49)	1.78(0.33)	0.52*(0.34)	29.4
Monocytes	0.54(0.13)	0.39(0.05)	0.15*(0.09)	37.5
Eosinophils	0.14(0.07)	0.16(0.08)	-0.02(0.02)	-11.6
Basophils	0.035(0.01)	0.027(0.007)	0.007*(0.004)	28.8

All values are mean(s.d.); * $P < 0.05$

Table 2. Red cell data and related indices, with overnight changes

	Evening	Morning	Differences
RBC × 10 ¹² /l	4.60(0.18)	4.67(0.26)	-0.07(0.10)
Hb, g/dl	13.53(0.56)	13.67(0.71)	-0.15(0.29)
MCV, fl	88.2(2.11)	89.5(2.1)	-1.33*(0.47)
MCH, pg	29.4(0.61)	29.3(0.74)	0.19(0.19)
MCHC, %	33.4(0.41)	32.7(0.28)	0.73*(0.31)
PCV, %	40.6(1.8)	41.7(2.3)	-1.1*(0.9)

All values are mean(s.d.); **P* < 0.05

than in the morning, as was the PCV. The MCH was normal, and unchanged overnight; the MCHC was slightly higher in the evening. All these small differences were statistically significant in a paired *t* test, and were consistent with the cells being slightly shrunk at the end of the day, and expanding again overnight, a volume change of the order of 2% being involved.

Reference ranges based on morning samples gathered

As the 'normal' range in the population at large encompasses people in a wide variety of conditions and situations, 'normal' values in haematology have no clear boundary against which abnormality may be judged. In *Table 3*, we present our own 'normal' haematological data, using the morning samples only, worked out as reference ranges (i.e. from mean(-2 s.d.) to mean(+2 s.d.); the mean observed is shown in bold type in the middle of this range), alongside the general population reference ranges published in a standard haematology textbook⁷. There was undoubtedly some skew in the distribution of the limited amount of data we have observed, particularly where cell counts were very low, so that assuming a symmetrical distribution about the observed mean in order to construct a reference range (±2 s.d.) is an oversimplification; hence the vanishingly small lower limit calculated for eosinophils.

Discussion

In general, the haematological characteristics of this highly trained group of competitive cyclists fell into a narrow band, and remained so even in the evening samples, at which time it was still possible to observe some of the effect of a stressful day's racing, this having ended, on average, about 6 h earlier than the evening sampling time.

In general, the PCV, red cell count, and haemoglobin concentration were all rather low. These are normal findings in highly trained racing cyclists, and the values for these three variables resemble those reported previously⁸. As there is no reason to suppose that there is any traumatic loss of red cells during cycling (as might be inferred if such low values were to be seen in a study of marathon runners, for instance), to have low-viscosity blood may be a physiological adaptation to the heat stress of cycling. It is certainly necessary to perfuse a large bed

Table 3. Reference ranges based on morning samples

	Racing cyclists	General population
Leucocytes × 10 ⁹ /l	(3.4) 5.04 (6.7)	(4.0) 7.5 (11.0)
Neutrophils × 10 ⁹ /l	(1.4) 2.52 (3.6)	(2.0) 4.75 (7.5)
Lymphocytes × 10 ⁹ /l	(1.1) 1.78 (2.5)	(1.5) 3.75 (4.0)
Monocytes × 10 ⁹ /l	(0.2) 0.39 (0.5)	(0.2) 0.5 (0.8)
Eosinophils × 10 ⁹ /l	(<0.01) 0.16 (0.32)	(0.04) 0.22 (0.4)
Basophils × 10 ⁹ /l	(0.01) 0.027 (0.04)	(<0.01) 0.05 (0.1)
RBC × 10 ¹² /l	(4.1) 4.67 (5.2)	(4.5) 5.5 (6.5)
Haemoglobin, g/dl	(12.2) 13.67 (15.1)	(13.0) 15.5 (18.0)
PCV, %	(37.0) 41.7 (47.0)	(40.0) 47.0 (54.0)
MCV, fl	(85.0) 89.5 (93.0)	(76.0) 86 (96)
MCH, pg	(27.8) 29.3 (30.8)	(27.0) 29.5 (32.0)
MCHC, %	(32.1) 32.7 (33.3)	(30.0) 32.5 (35.0)

of dilated skin blood vessels during a long-distance multi-stage race of this type, and plasma volume is expanded both by training and by heat acclimation⁹. A similar haemodilution normally occurs in pregnancy, again probably to assist with heat dissipation as the growing embryo imposes an extra heat load on its mother. This haemodilution might, of course, account for a fraction of the tendency of each of the leucocyte counts to be low (if it is supposed that the body attempts to regulate total circulating cell numbers of each type and plasma volume as an independent variable, with no monitoring of the 'concentration' of cell types). During the 12 racing days studied, the morning mean red cell count in the cyclists was 85% of the textbook reference value. Total and individual leucocyte counts were, however, relatively lower than this; the total count being 67%, the neutrophil count 53%, and the lymphocyte count 47% of the respective reference mean values as given in *Table 3*.

The red cells of the evening samples were slightly (<2%) shrunk, recovering their volume overnight. Shrinkage in the evening samples is unlikely to be a consequence of the longer period in storage before counting; in general, red cells swell if counting is delayed. MCV has previously been shown to fall during exercise, and to recover slowly, the initial shrinkage being associated with dehydration induced by exercise¹⁰.

The levels of white blood cells are clearly slightly low in comparison with those of the general population. It has been shown that no change occurs in the white cell counts of samples anti-coagulated by K-EDTA during storage in a refrigerator (at an unspecified temperature) for 24 h¹¹. There was some evidence of a left-shift among the granulocytes in our samples (meaning that the nucleus was less lobulated and indented, which implies youthfulness among the circulating population of cells) which may have a shorter half-life in these very active young men. This also suggests that a slightly low cell count is a genuine result, and was not due to technical artefacts.

Most of the leucocyte levels in the evening samples, as compared to the morning samples (neutrophils, lymphocytes, monocytes, and basophils) were elevated. While a relative neutrophilia is frequently and usually reported in delayed leucocytosis

sis, this almost general increase in leucocytes (eosinophils excepted) differs from the usual pattern seen as a response during recovery from more 'normal' amounts of exercise. Soon after shorter periods of heavy exercise (e.g. periods of up to 1 h), at about 2–6 h into recovery, a lymphopaenia has been reported^{4,12}. However, in all respects, the more general leucocytosis we observed about 6 h after this more prolonged heavy exercise (these races took about 4 h per day) was of modest degree, and one must also consider the possibility of seeing diurnal changes (due to circadian rhythms) when samples are taken 12 h apart.

The sampling times that we used would not necessarily catch diurnal peaks and troughs. For eosinophils, it is likely that the samples were taken as the count was still falling in the morning, and rising again in the evening, leaving the observed values little different; this is the expected outcome given the times of sampling used in relation to the normal diurnal rhythm. There is a well-described diurnal rhythm for eosinophils^{13,14}, with a trough at 1000 h and a peak at or after midnight (2400–0400 h). There is a less convincingly-documented rhythm for basophils which seems to be generally similar¹⁵; we have found very little published data on these cells. We observed an increased level in the evening in this case. Both of these cell types, along with lymphocytes, appear to be depressed by rising cortisol levels, but to recover in the night when cortisol levels are low¹⁶. The evening level of lymphocytes in our study was elevated relative to the morning level by a change of the same order of magnitude as that reported as a simple diurnal rhythm in normal subjects¹⁷, in which a clear trough in lymphocyte level was found at 0800 h. Similar findings have been reported by earlier workers making studies without the benefit of automatic cell counters^{18,19}, and by many more recent studies, summarized by Lévi *et al.*²⁰. The diurnal rhythm for monocytes is at its peak at 2000 h, at which time Haus *et al.*²¹ found the level to be approximately 50% higher than that in the early morning, so here again the relative difference of the results we have observed fulfils expectations on the basis of a simple diurnal variation.

In sedentary subjects, neutrophils were also found to peak late in the day, at 1600–2000 h, by Haus *et al.*²¹, and then to fall, a rhythm which is almost directly out of phase with the cortisol level. This hormone reaches its nadir at midnight, but in sedentary man it is already quite low by 2000 h²². We have no reason to attribute the sustained relatively high neutrophil count in our evening samples to a current difference in cortisol level, but the result, despite the exercise recently performed, does follow the normal diurnal pattern.

Although all these samples gave white cell counts within the reference range used for the general population, we suggest that clinical judgements of white cell levels during such an athletic event could best be made by taking a morning sample, and then expecting most of the data to fall towards the lower of the appropriate normal reference range. There may be a small degree of 'immunosuppression' expressed in these terms (as cell counts) in such highly trained

subjects, although in the absence of knowledge of turnover rates of the cell populations, this might easily be an erroneous interpretation to place upon the counts observed. It is also clear that one should not expect the normal average, let alone high, haemoglobin or haematocrit (PCV) values in these athletes; while this is not an original observation, it is a point that bears repetition: a spot check blood sample can easily give rise to unnecessary speculation about a 'near-anaemic' condition. Guglielmini *et al.*⁸ reported that monthly intravenous iron supplementation made no difference to the relatively low haematocrit (PCV) in professional cyclists, although their subjects' iron stores (as represented by plasma ferritin levels) were then clearly elevated.

Acknowledgements

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Dr Gerry Haggerty MRCP Dip. Sports Medicine,
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- 10.30 am **"Basics of Sports Injury Diagnosis"**
Dr Faith Gardner DRCOG, DIP Sports Medicine
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- 11.30 am Coffee
- 12.10 pm **"Soft Tissue Injury Prevention"**
Warm-up, Stretching & Cool Down
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- 1.00 pm Lunch
- 2.25 pm **"The Role of Nutrition in Sports Performance"**
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- 3.20 pm **"Gender Differences in Sport"**
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Mr Alan Hodson, MA MCSP Assistant Director of
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- 11.20 am Coffee
- 12.00 noon **"Stress Fractures"**
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David Rees M.Ch.Orth.FRCS Leighton Hospital and
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- 5.40 pm Close

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