Diurnal Variations of Blood Constituents in Young Thoroughbred Horses

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To delineate points to be attended to in utilizing blood constituents as parameters for diagnostic examination of race horses, serial changes of blood constituents were followed on a day of resting and a day of physical exercise in five 2-year-old Thoroughbred horses (2 males and 3 females) currently under a course of training equivalent to that for young prospective race horses. Of 31 blood constituent parameters assessed which are commonly measured in the clinical setting, 27 parameters revealed significant within-day variations ($P < 0.05$). Variations by 20% or more which are presumed to be of practical value from their nature were observed for 14 parameters: total leukocyte counts, neutrophil counts, lymphocyte counts, eosinophil counts, and serum levels of lactate dehydrogenase, glutamic dehydrogenase, total bilirubin, uric acid, glucose, triglyceride, potassium, inorganic phosphorus, triiodothyronine and thyroxine. Whilst the principal source of variations was physical exercise, there were several parameters which were inferred to be affected by such factors as feeding and daytime/night. Thus the present data indicated importance of due consideration of feeding conditions including quality and amount of physical exercise and of blood sampling time, for more accurate interpretation of data of hematologic and blood chemical tests.

Key words: diurnal variation, blood constituents, resting day, exercise day

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A wide variety of blood constituents are measured as parameters to assess training conditions as well as in the clinical diagnosis of race horses. However, it is often difficult to determine the blood sampling time since race horses are assigned a hard physical exercise nearly every day during relatively early hours and that they possess many factors likely to strongly affect blood constituents, e.g. the relatively long alimental tract and intracecal digestion.

It is often the case, in practice, that collection of blood specimens is carried out at the most convenient time under a given feeding condition or at any time on an emergency. Analysis of the data and determination of whether or not the data obtained are within the physiological limits of variation entail difficulty in no few instances. This study represents an attempt to find solution of this

problem by serially assessing blood constituent parameters, especially those commonly determined in clinical laboratory examination for changes on a day of resting and a day of physical exercise in young Thoroughbred horses currently under a course of training equivalent to that for young prospective race horses.

Materials and Methods

Five healthy, 2-year-old Thoroughbred horses (2 colts and 3 fillies) currently on a training program at the Race Horse School of the Japan Racing Association were used as subjects of this study.

The experiment was performed on August, 1992, a single weekly resting day in stalls (rest day) and on a day with an approximately one-hour exercise consisting of running at full speed along a 1000-meter dirt course (exercise day). Blood sam-

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pling was made at 4-hour intervals over a period of 24 hours beginning at 7:30 a.m. The full-speed running exercise commenced at 10:00 a.m. and lasted for about one hour, and animals remained in their respective boxstalls throughout the rest of that day. Feeding was made four times a day (at 6:30, 11:20, 16:20 and 19:20), the ration being the least at 11:20 and that at 19:20 comprising hay alone.

Blood was obtained within the stall through the jugular vein using an evacuated blood collection tube containing ethylene-diamine-tetra-acetic acid (EDTA) dipotassium salt solution and a plain evacuated tube. Erythrocyte counts (RBC) and total leukocyte counts (WBC) were determined with an automated blood cell counter. Packed cell volume (PCV) was measured by the hematocrit method. The number of eosinophils (Eo) were determined with Tatai's eosinophil counting plate. Enumeration of neutrophils (Ne) and lymphocytes (Ly) was indirectly made from their differential counts and total leukocyte counts. Serum samples were assessed for biochemical parameters with a Hitachi 736 Autoanalyzer (Hitachi Ltd., Tokyo); the parameters included creatine phosphokinase (CPK), γ -glutamyl transpeptidase (γ -GTP), aspartate aminotransferase (AST), glutamic dehydrogenase (GLDH), lactate dehydrogenase (LDH), cholinesterase (ChE), alkaline phosphatase (ALP), creatinine (Cre), albumin (Alb), total protein (TP), total bilirubin (TB), blood urea nitrogen (BUN), uric acid (UA), glucose (Glu), total cholesterol

(T-Cho), triglyceride (TG), phospholipid (PL), Na, K, Cl, Ca, Fe and inorganic phosphorous (IP). Two thyroid hormones in serum, triiodothyronine (T₃) and thyroxine (T₄), were quantitated by enzyme immunoassay.

The above hematologic and blood biochemical parameters at the consecutive blood sampling times were assessed for changes and evaluated using the GLM Procedure of the Statistical Analysis Systems Institute (SAS) [7] and two-way analysis of variance.

Results

Statistically significant changes with time ($P < 0.05$) were observed for 27 of the 31 parameters examined (Table 1). These 27 parameters showing significant changes with time were classified into three according to the magnitude of changes, i.e. 1) $< 10\%$, 2) 10 to $< 20\%$, and 3) $\geq 20\%$, in Table 2. Figs. 1 to 3 illustrate the time-course of changes of only those parameters which showed 20% or greater within-day changes on either the exercise day or the rest day.

Diurnal variations of blood cell parameters were noted more frequently on the exercise day. Total leukocyte counts and neutrophil counts began to increase following exercise and showed maximum increases by about 25 and 40%, respectively, in a few hours in the afternoon. Eosinophils, conversely, showed a nadir at that time. Lymphocyte counts

Table 1. Parameters showing significant changes with time among the 31 parameters assessed

Parameters	Rest day	Exercise day	Parameters	Rest day	Exercise day
RBC	N.S.	N.S.	TB	*	**
WBC	N.S.	**	UA	**	**
PCV	N.S.	N.S.	BUN	*	**
Eo	N.S.	*	Glu	**	**
Ne	N.S.	**	T-Cho	**	**
Ly	**	**	TG	**	N.S.
CPK	N.S.	N.S.	PL	**	**
γ -GTP	N.S.	**	Na	**	**
AST	N.S.	**	Ca	*	N.S.
GLDH	**	**	K	**	**
LDH	**	**	Fe	N.S.	N.S.
ChE	**	N.S.	Cl	N.S.	**
ALP	**	**	IP	**	**
TP	**	**	T ₃	N.S.	**
Cre	**	**	T ₄	**	**
Alb	**	**			

N.S.: No significant difference. *: $P < 0.05$. **: $P < 0.01$.

Table 2. Classification of parameters according to rate of variation

1) <10%	ALP, TP, Alb, Cre*, T-Cho*, Na, Cl*, Ca**
2) 10% to <20%	AST*, LDH*, γ -GTP*, ChE**, TB**, BUN, Cre**, T-Cho**, PL, T ₄ **
3) $\geq 20\%$	WBC*, Ne*, ly, Eo*, LDH**, GLDH, UA, TB*, Glu, TG**, K, IP, T ₃ *, T ₄ *

* Only on exercise day. ** Only on rest day.

exhibited a different trend of change; the cells increased by 20 to 30% nocturnally on both the rest day and the exercise day.

Of the serum biochemical parameters examined, the majority displayed changes on both

the rest day and the exercise day. Generally, a more conspicuous transient change occurred following exercise. GLDH and uric acid levels were nearly 4 times as high after exercise, compared to pre-exercise values. For LDH, there were greater change on the rest day than on the exercise day. Several other parameters revealed variations much less related to exercise. Serum glucose level showed a peak postprandially in the morning whereas peak serum K and IP levels were observed at night. Serum triglyceride level showed increases only on the rest day.

Fig. 1. Changes in hematologic parameters. Each graph depicts time-course of mean and standard deviation over the indicated times. Open circles show values on rest day and closed circles those on exercise day.

Fig. 2. Changes in serum biochemical parameters (1). See legend for Fig. 1.

Fig. 3. Changes in serum biochemical parameters (2). See legend for Fig. 1.

Discussion

The present data demonstrated significant changes with time of the majority of the hematologic and blood biochemical parameters, particularly in association with exercise. The pattern of response, nevertheless, varied considerably with parameters.

Total leukocyte counts, neutrophil counts and eosinophil counts showed a peak or a nadir 5 hours after exercise. The increase of neutrophils and the consequent increase of total leukocyte counts might result from 1) drawing of leukocytes from the bone marrow, spleen, lungs and other

organs into bloodstream due to increase in circulating blood volume, 2) shift of leukocytes from the marginal pool in response to plasma catecholamine elevation, and 3) stimulation of neutrophil production by increased cytokines such as interleukin 1. When viewed with reference to the response time observed, 3) was inferred to be most accountable as a contributory factor [3, 5]. The decrease of eosinophil counts was consequent on the exercise stress [6] and the present data indicate that the relevant response appears in the peripheral blood with a few hours' time lag.

GLDH and uric acid are liberated from skeletal muscle cells into circulating blood rapidly in

response to exercise [4, 10]. The present results show that the blood sampling time should be taken into account in interpreting data on serum level of GLDH which remains long in bloodstream, unlike uric acid which is eliminated into urine in a short time [4]. Similar responses of the two thyroid hormones (T3 and T4) to exercise stimuli, though modest in magnitude of change, were also evident in the present series. This would indicate that the pattern of variation should be considered in the analysis of their responses.

Of the various serum enzyme level examined, LDH alone displayed a greater increase on the rest day. This might imply a delayed peak of serum LDH as a response to exercise [2, 9]. No such phenomenon was observed, however, with AST which has been reported to show a similar pattern of variation [2,9]. Serum CPK level, which is generally recognized to rapidly and keenly reflect muscular damage, did not show any appreciable change even on the exercise day. Thus the exercise practiced seemed unlikely to be so vigorous as to be reflected in this respect in the horses. The serum LDH elevation seen on the rest day suggest either a higher sensitivity of this enzyme to exercise stimuli than CPK, or effects of secondary changes, particularly kinetogenic hemolysis presumed from the increased total bilirubin and an associated effect on the kidneys. These are yet to be investigated in detail.

Peripheral blood lymphocyte counts showed an increase at night on both the rest day and the exercise day; hence probably little related to exercise. A similar trend has been observed in humans [8] and attributed to occurrence of maximal response of cellular immunity in the inactive phase.

On the rest day, serum triglyceride concentration increased after feeding and remained increased for more than 10 hours. This finding stresses importance of attention to be paid to postprandial lapse of time as in humans [1] in analyzing serum triglyceride concentration data. Such variations were not existent on the exercise day, suggesting enhanced energy metabolism. This would add importance of considering the nature of exercise practice besides the feeding time.

From the above results and discussion and with the exception of nocturnal variations which are of infrequent use, it follows that physiological pa-

rameters that require due consideration of conditions of sample collection in the clinical laboratory assessments of race horses, include post-exercise total leukocyte counts, neutrophil counts, serum GLDH and serum uric acid, morning postprandial serum glucose, and rest day serum LDH and triglycerides. While the rest of the parameters were found to be not so overtly affected by physical exercise or feeding, it is considered to be of importance to maintain the animal as much rested as possible in collecting specimens for clinical laboratory tests.

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