Plasma Catecholamine, Adrenocorticotropin and Cortisol Responses to Exhaustive Incremental Treadmill Exercise of the Thoroughbred Horse

Masahiko KUROSAWA1*, Shun-ichi NAGATA1, Fujie TAKEDA1, Kyousuke MIMA1, Atsushi HIRAGA2, Makoto KAI2 and Kazuyoshi TAYA3

1Research Department, Laboratory of Racing Chemistry, 4–37–6 Kamiyoga, Setagaya-ku, Tokyo 158-0098, 2Equine Research Institute, Japan Racing Association, 321–4 Tokami-cho, Utsunomiya, Tochigi 320-0856 and 3Laboratory of Veterinary Physiology, Tokyo University of Agriculture and Technology, 3–5–8 Saiwai-cho, Fuchu, Tokyo 183-0054, Japan

Plasma adrenaline (Ad), noradrenaline (NA), adrenocorticotropin (ACTH) and cortisol responses to exhaustive incremental treadmill exercise were investigated in seven Thoroughbreds. Blood lactate (LA), heart rate (HR) and oxygen uptake (VO2) were measured and their correlations with plasma hormone levels were examined. Although the magnitude of plasma Ad, NA and ACTH response to the exercise was great, that of cortisol was very slight. Plasma Ad and NA levels reached peaks at exhaustion in all Thoroughbreds. On the other hand, plasma ACTH levels reached peaks at a different time in each Thoroughbred, followed by a 10–15 min delayed peak of plasma cortisol levels. The maximal levels of plasma Ad, NA, ACTH and cortisol were significantly 300, 150, 70 and 1.8 fold higher, respectively, than the pre-exercise levels. Plasma Ad, NA and ACTH during exercise were highly and significantly correlated to each other. The noticeable increase in plasma Ad levels during exercise was characteristic of Thoroughbreds. Plasma Ad, NA and ACTH responses during exercise were significantly correlated with treadmill velocity, blood LA, HR and VO2 in an exponential manner. The threshold for blood LA was significantly lower than the threshold for Ad, NA and ACTH. In conclusion, the present data on plasma CA and ACTH responses to exercise suggest that the sympathoadrenal axis and the pituitary gland are highly activated by exercise in Thoroughbreds. In addition, it is suggested that plasma CA and ACTH levels as well as LA and HR may be useful indicators for estimating exercise stress and physical fitness in the Thoroughbred.

Key words: adrenocorticotropin, catecholamines, cortisol, treadmill exercise, Thoroughbred

It has been demonstrated that plasma levels of catecholamines (CA) of adrenaline (Ad) and noradrenaline (NA) increase with the intensity and the duration of exercise [10, 14]. Plasma levels of Ad and NA, which reflect the sympathoadrenal activity, are useful indicators for estimating exercise stress, physical fitness and training effect as well as blood lactate (LA) and heart rate (HR) in humans [9, 15, 18]. In addition, plasma CA responses to exercise have been correlated with cardiorespiratory variables, such as LA, HR and oxygen uptake (VO2) [12, 18, 26]. Snow et al. [29] reported that much greater responses in plasma CA during exercise were observed in horses than humans, but the correlation between plasma CA responses and cardiorespiratory variables remains unclear. On the other hand, plasma ACTH levels also increase with the degree of exercise [8, 16]. Adrenocorticotropin (ACTH) stimulates the production of cortisol from the adrenal cortex in mammals. Accordingly, an exercise-induced increase in plasma ACTH is followed by an increase in plasma cortisol levels [4, 8, 16]. Although the exercise-induced activation of the adrenocortical system has also been evidenced by significant increases in plasma cortisol in horses as in the
case of humans [20, 23], plasma ACTH responses to exercise in horses remain to be determined.

The purpose of this study was to investigate plasma Ad, NA, ACTH and cortisol responses to exhaustive incremental treadmill exercise in Thoroughbreds. The correlation among plasma levels of these hormones and cardiorespiratory variables, such as LA, HR and VO₂, in exercising Thoroughbreds were also investigated.

Materials and Methods

Animals
Seven healthy, trained Thoroughbreds ranging in age from 4 to 5 years with an average weight of 450 ± 11 kg were used in this study. All horses were acclimatized to exercise on a high-speed equine treadmill (Mustang-2200, Kagra, Switzerland). Prior to the incremental treadmill exercise test, each horse was trained on a treadmill 5 days per week for a month.

Experimental preparation
On the day of the study, a 14-gauge angiocatheter was aseptically placed into a right jugular vein before exercise. The catheter was heparinized and was affixed to the skin with sutures. An extension line was attached to the catheter for blood sampling during exercise. Self-adhesive electrocardiogram patches were attached for HR determination.

Exercise protocol
The incremental exercise tests were performed according to the exercise protocol described by Seeherman et al. [28]. Following 5 min of warm-up exercise at 4 m/s and 5 min rest on a treadmill at a 0% incline, the treadmill was then inclined to a 10% slope and the exercise test was started at 1.8 m/s. The velocity of the treadmill was then sequentially increased in 1 min intervals to 2.7, 3.4, 4.5, 5.4, 6.8, 9.0, 9.8, 10.8 and 11.6 m/s. Each horse completed the incremental exercise until exhaustion when the horse could not keep up with the treadmill. Maximal intensity of treadmill work was defined as the treadmill velocity at exhaustion. During the incremental exercise test, VO₂ was continuously measured by means of an open flow respirometry system [7] and determined during the final 30 sec of each step. The highest level of VO₂ was defined as the VO₂ peak. The HR was continuously recorded during exercise with a heart rate monitor (Bandage-XL, Pollar, Finland) and calculated during the last 30 sec of each step. After completion of the incremental exercise, the horses were walked at 1.0 m/s for 15 min at a 0% incline.

Blood sample collection
Blood samples were collected via the venous catheter at rest prior to exercise, immediately before (WP) and after warming up, and during the last 20 sec of each step of the exercise. After completion of the exercise also, blood was collected at 1 (A1), 5 (A5), 15 (A15), 60 (A60) and 120 (A120) min in prechilled tubes containing EDTA-2Na for the assay of CA, and heparin sodium and 500 IU of aprotinin per 1 ml of blood for the assay of ACTH and cortisol. All blood samples were kept ice-cold and were centrifuged at 3,500 g for 10 min at 4°C. Plasma was stored frozen at -40°C until analysis.

Biochemical analyses
Plasma Ad and NA levels were determined by a high-performance liquid chromatographic method with electrochemical detection [17]. The limit for both detections was 10 pg/ml. The intra-assay coefficients of variation were less than 5%. Plasma cortisol and ACTH levels were determined by the specific radioimmunoassay method for each hormone [1, 33]. The detection limits for cortisol and ACTH were 2 ng/ml and 5 pg/ml, respectively. The intra-assay coefficients of variation were 7.7% for cortisol and 4.7% for ACTH. Blood LA levels were determined with a lactate analyzer (YSI-1500, Yellow Spring Instruments, Ohio, USA). Packed cell volume (PCV) was measured with a microhematocrit centrifuge.

Thresholds for blood LA (LT), plasma Ad (Ad-T), NA (NA-T) and ACTH (ACTH-T) detection
LT, Ad-T, NA-T and ACTH-T were determined as VO₂ corresponding to the individual breaking point of each curve calculated by the two-segmental linear regressions previously described [3].

Statistical analyses
All data are presented as the mean ± SE. The effects of exercise were evaluated by a one-way analyses of variance (ANOVA) with repeated measures. Because plasma levels of Ad, NA and ACTH at some points in time had skewed distributions, analyses were made after logarithmic transformations. If the ANOVA indicated an effect of exercise, a Tukey post-hoc procedure was applied. Ad-T, NA-T and ACTH-T were compared with LT by Student’s paired t-tests. The maximal levels in plasma hormones, LA, PCV and HR were compared with pre-exercise levels by Student’s paired t-tests. The significance of associations between variables was determined by calculation of the correlation coefficients and least-squares linear regression
analysis, with plasma hormonal levels logarithmically transformed in exponential correlation.

**Results**

The maximal intensity of treadmill work was 10.5 ± 0.3 m/s. Although each horse performed exercise to exhaustion at a different step, all of the horses completed the 9.0 m/s step of the treadmill test. Details of the plasma Ad, NA, ACTH and cortisol responses induced by exhaustive incremental exercise are shown in Table 1. The data for the statistical analysis during exercise were obtained at 1.8 m/s, 9.0 m/s and exhaustion. The plasma levels of Ad and NA significantly increased throughout the incremental exercise as compared with the pre-exercise levels (P<0.01 for both), and reached a peak exactly at exhaustion in all Thoroughbreds. The peak values were significantly higher than at other times. The maximum levels of plasma Ad and NA significantly increased to about 300 times (P<0.01) and 150 times (P<0.01), respectively, as compared with the pre-exercise levels (Table 2). They rapidly declined after exercise with an approximately 50% reduction from the peak levels occurring at A1. The significant exercise-induced increase in the plasma Ad and NA levels was observed until A5 and A15, respectively.

Plasma ACTH levels significantly increased during the incremental exercise as compared with the pre-exercise levels. They had two peaks and their values were not necessarily higher than at other times. Plasma ACTH levels reached peaks at exhaustion for three Thoroughbreds and at A5 for four Thoroughbreds. The maximal level of plasma ACTH was a significant (P<0.01) approximately a 70-fold increase as compared with the pre-exercise level (Table 2). ANOVA revealed a significant effect of the exercise on plasma cortisol, but the plasma cortisol responses to exhaustive incremental exercise were not as dynamic as the plasma CA and ACTH responses. Plasma cortisol levels reached peaks at exhaustion for two Thoroughbreds and at A15 for five Thoroughbreds. Maximal levels of plasma cortisol increased only 1.8-fold (P<0.01) as compared with the pre-exercise levels (Table 2). The plasma levels of ACTH and cortisol then returned to the levels before exercise at A120.

Correlations between plasma hormonal levels and cardiorespiratory variables during incremental exercise are shown in Figs. 1–4. Exercise-induced responses in

### Table 1. Changes in plasma hormone response to exhaustive incremental treadmill exercise in Thoroughbreds

<table>
<thead>
<tr>
<th></th>
<th>Adrenaline (pg/ml)</th>
<th>Noradrenaline (pg/ml)</th>
<th>ACTH (pg/ml)</th>
<th>Cortisol (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre</td>
<td>53.0 ± 3.8a</td>
<td>85.8 ± 10.6a</td>
<td>30.9 ± 5.1a</td>
<td>36.5 ± 8.6</td>
</tr>
<tr>
<td>WP</td>
<td>95.3 ± 11.1a</td>
<td>106 ± 14a</td>
<td>39.2 ± 9.6a</td>
<td>34.6 ± 5.6</td>
</tr>
<tr>
<td>1.8 m/s</td>
<td>293 ± 74b</td>
<td>219 ± 28b</td>
<td>78.0 ± 18.7ac</td>
<td>39.2 ± 7.2</td>
</tr>
<tr>
<td>9.0 m/s</td>
<td>3165 ± 1096d</td>
<td>4300 ± 1576d</td>
<td>570 ± 191d</td>
<td>47.1 ± 7.7</td>
</tr>
<tr>
<td>Exhaustion</td>
<td>16227 ± 4364e</td>
<td>13046 ± 3880e</td>
<td>1477 ± 535e</td>
<td>57.2 ± 18.9</td>
</tr>
<tr>
<td>A1</td>
<td>6085 ± 1536d</td>
<td>8449 ± 3942d</td>
<td>1210 ± 392d</td>
<td>48.2 ± 8.9</td>
</tr>
<tr>
<td>A5</td>
<td>669 ± 159e</td>
<td>2372 ± 1792e</td>
<td>1859 ± 522e</td>
<td>46.3 ± 7.6</td>
</tr>
<tr>
<td>A15</td>
<td>246 ± 53b</td>
<td>256 ± 37b</td>
<td>1019 ± 278e</td>
<td>53.9 ± 9.0</td>
</tr>
<tr>
<td>A60</td>
<td>73.4 ± 10.5a</td>
<td>92.4 ± 10.8a</td>
<td>105 ± 28a</td>
<td>47.6 ± 9.4</td>
</tr>
<tr>
<td>A120</td>
<td>66.4 ± 8.1*</td>
<td>87.5 ± 10.8a</td>
<td>18.3 ± 3.5ab</td>
<td>38.5 ± 8.4</td>
</tr>
</tbody>
</table>

Values are the means ± SE. Pre: at rest pre exercise, WP: immediately before warm-up, A1, A5, A15, A60 and A120: 1, 5, 15, 60, 120 min after exercise, respectively. Values without a common letter are significantly different (P<0.05).

### Table 2. Maximal responses in plasma catecholamines, ACTH and cortisol to exhaustive incremental treadmill exercise

<table>
<thead>
<tr>
<th></th>
<th>Adrenaline (pg/ml)</th>
<th>Noradrenaline (pg/ml)</th>
<th>ACTH (pg/ml)</th>
<th>Cortisol (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pre-exercise values</td>
<td>53.0 ± 3.8</td>
<td>85.8 ± 10.6</td>
<td>30.9 ± 5.1</td>
<td>36.5 ± 8.6</td>
</tr>
<tr>
<td>maximal values</td>
<td>16227 ± 4364**</td>
<td>13046 ± 3880**</td>
<td>2132 ± 493**</td>
<td>66.8 ± 18.3**</td>
</tr>
</tbody>
</table>

Values are the means ± SE. **Significant difference (P<0.01) from pre-exercise values.
Fig. 1. Relationship between treadmill velocity and plasma adrenaline (a), noradrenaline (b), adrenocorticotropin (ACTH) (c) and cortisol (d). Values in parentheses are the means ± SE of correlation coefficients for seven Thoroughbreds.

Fig. 2. Relationship between blood lactate and plasma adrenaline (a), noradrenaline (b), adrenocorticotropin (ACTH) (c) and cortisol (d). Values in parentheses are the means ± SE of correlation coefficients for seven Thoroughbreds.
Fig. 3. Relationship between heart rate and plasma adrenaline (a), noradrenaline (b), adrenocorticotropin (ACTH) (c) and cortisol (d). Values in parentheses are the means ± SE of correlation coefficients for seven Thoroughbreds.

Fig. 4. Relationship between oxygen uptake and plasma adrenaline (a), noradrenaline (b), adrenocorticotropin (ACTH) (c) and cortisol (d). Values in parentheses are the means ± SE of correlation coefficients for seven Thoroughbreds.
plasma Ad, NA and ACTH were significantly (P<0.001) correlated with treadmill velocity in an exponential manner (Fig. 1). They were also significantly (P<0.001) correlated with LA responses in an exponential manner (Fig. 2). In particular, a considerably high correlation (r>0.97) was observed between plasma CA and LA responses for each Thoroughbred. In addition, plasma CA and ACTH during incremental exercise were also correlated with HR and VO2, and increased abruptly at around 200 beats/min for HR and 150 ml/kg/min for VO2 (Figs. 3 and 4). In general, the correlation coefficients for each Thoroughbred were considerably high (above 0.9 in almost cases), although the correlation coefficients for all Thoroughbreds were below 0.9 due to marked individual variations in the plasma Ad, NA and ACTH responses. Plasma Ad, NA and ACTH responses to incremental exercise were highly and linearly correlated to each other and mean correlation coefficients for each Thoroughbred were above 0.94 (Fig. 5).

\[ y = 0.953x + 0.388 \\
\text{r} = 0.876 \\
P < 0.001 \\
(r: 0.990 \pm 0.007) \]

\[ y = 0.111x + 0.098 \\
\text{r} = 0.833 \\
P < 0.001 \\
(r: 0.963 \pm 0.012) \]

\[ y = 0.126x + 0.069 \\
\text{r} = 0.932 \\
P < 0.001 \\
(r: 0.941 \pm 0.016) \]

Table 3. Oxygen uptake at lactate, catecholamines, and ACTH thresholds in Thoroughbreds during exhaustive treadmill exercise

<table>
<thead>
<tr>
<th>LT</th>
<th>Ad-T</th>
<th>NA-T</th>
<th>ACTH-T</th>
</tr>
</thead>
<tbody>
<tr>
<td>VO2</td>
<td>116.3 ± 3.5</td>
<td>126.9* ± 3.9</td>
<td>125.9* ± 3.7</td>
</tr>
</tbody>
</table>

Values are the means ± SE. VO2: oxygen uptake, LT, Ad-T, NA-T, and ACTH-T: lactate, adrenaline, noradrenaline, and ACTH thresholds, respectively.

*Significant difference (P<0.05) from LT.
Table 4. Exercise-induced responses in oxygen uptake (\( \dot{V}O_2 \)), blood lactate (LA), packed cell volume (PCV) and heart rate (HR)

<table>
<thead>
<tr>
<th></th>
<th>( \dot{V}O_2 ) (ml/kg/min)</th>
<th>LA (mmol/l)</th>
<th>PCV (%)</th>
<th>HR (beats/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pre-exercise values</td>
<td>N.D.</td>
<td>0.44 ± 0.02</td>
<td>38.0 ± 0.9</td>
<td>32.4 ± 1.2</td>
</tr>
<tr>
<td>peak values following exercise</td>
<td>163.5 ± 4.1</td>
<td>16.57 ± 1.70**</td>
<td>57.4 ± 1.4**</td>
<td>224.1 ± 3.0**</td>
</tr>
</tbody>
</table>

Values are the means ± SE. N.D.: not determined. **: Significant difference (P<0.01) from pre-exercise values.

Discussion

The present study clearly demonstrated that the incremental exercise with a high-speed equine treadmill caused intensity-dependent increases in plasma CA and ACTH levels in an exponential manner. Plasma CA responses in exercising Thoroughbreds were much greater than those previously reported in humans and similar to those reported by Snow et al. [29] in the horse. In humans, plasma NA levels are three to five times greater than that for Ad for a given work intensity [14, 19, 22]. In the present study, the exercise-induced increase in plasma Ad in Thoroughbreds was similar to that for NA as previously reported [29]. Ad primarily originates from the adrenal medulla [12, 15]. Furthermore, changes in the plasma Ad levels reflect changes in secretion rather than in clearance [15]. Accordingly, plasma Ad levels reflect Ad secretion from the adrenal medulla. It is probable that the greater increase in the plasma Ad in exercising Thoroughbreds may indicate higher activation of the adrenal medulla. Supporting the present findings, Snow et al. [29] suggested that the adrenal medulla may play a more important role in modulating exercise-induced physiological responses in Thoroughbreds than in humans. Plasma NA levels reflect the exercise-induced activation of the sympathetic nervous system [11, 22]. Although the origin of circulating NA in the horse has not been clarified yet, plasma NA levels in the horse may result from not only neuronal spillover from active sympathetic fibers but also release from the adrenal medulla as previously reported [29]. The horses used in this study were well trained with the much higher peak values of \( \dot{V}O_2 \), HR and PCV than humans. The equine has almost 3 times a greater \( \dot{V}O_2 \)max than human athletes [25]. The Thoroughbred is an excellent athlete and its high \( \dot{V}O_2 \)max is caused by a wide range of HR response together with a dramatic increase in PCV, which is induced by splenic contraction sympathetically activated by exercise [30]. During exercise, an increase in sympathoadrenal activity is the most important autonomic neuroendocrine response. If sympathetic activity is impeded, exercise capacity is reduced [6, 31]. Accordingly, the present data on plasma CA responses to exercise suggest that the high activation of the sympathoadrenal axis during exercise greatly contributes to the superiority of athletic performance in Thoroughbreds.

It has been documented that Ad is a strong activator of muscle glycogenolysis through the action of the \( \beta \)-adrenergic receptors [32]. Jansson et al. [13] have reported that infusion of Ad may enhance muscle glycogenolysis during submaximal exercise. On the other hand, Schneider et al. [26] hypothesized that the reduced blood and muscle pH accompanying LA production may have been the cause of circulating CA increase during exercise. Plasma CA responses were highly and significantly correlated with blood LA responses during exercise in an exponential manner in Thoroughbreds but in a linear manner in humans [12, 18]. In addition, LT is significantly lower than Ad-T and NA-T during exercise in Thoroughbreds. From these results, it is probable that plasma Ad and NA may behave according to blood LA. Accordingly, our results support the hypothesis that a decrease in muscle pH, due to increased LA production, stimulated a reflex increase in sympathetic outflow and a subsequent rise in CA levels [26, 35].

It has been known that the ACTH secretion from the pituitary, which is stimulated by arginine vasopressin and corticotropin-releasing hormone, stimulates the production of glucocorticoids from the adrenal cortex [2, 21]. In both humans and horses, the most important glucocorticoid is cortisol. Therefore, in order to estimate the pituitary-adrenal response to exercise, plasma ACTH and cortisol responses have been extensively studied and the exercise-induced increase in plasma ACTH and cortisol have been reported in humans [4, 8, 16, 21, 27] and horses [5]. But, in most studies, plasma ACTH and cortisol responses have only been estimated by comparing pre-exercise levels with post-exercise levels. In the present study, plasma ACTH and cortisol
responses to exhaustive incremental exercise were investigated during exercise and recovery. As a consequence, plasma ACTH levels during exercise exponentially increased with exercise intensity and reached peaks at exhaustion for three Thoroughbreds and at A5 for four Thoroughbreds. The incremental exercise to exhaustion during about 9 min produced approximately a 70-fold increase in plasma ACTH. On the other hand, plasma cortisol levels, which increased only 1.8 fold in response to exercise, reached a peak at exhaustion for two Thoroughbreds and A15 for five Thoroughbreds. Plasma ACTH levels reached peaks followed by a delayed increase in plasma cortisol in Thoroughbreds as well as in humans [4, 16, 27]. Although the plasma ACTH response to exhaustive incremental exercise in Thoroughbreds was much greater than that in humans, the plasma cortisol response was similar in both Thoroughbreds and humans [27]. This indicates that a great deal of circulating ACTH induced by exercise may not stimulate cortisol release from the adrenal cortex in Thoroughbreds. The adrenocortical response may be limited by the cortisol production ability in the adrenal cortex. It is also possible that exercise may increase immuno-reactive ACTH-like substances, which may have less bioactivity to the production of cortisol from the adrenal cortex.

A high and significant correlation between plasma ACTH and blood LA responses to exercise was observed. LT was significantly lower than ACTH-T, which was almost similar to Ad-T and NA-T. These results support the assumption that LA production is important to the activation of the pituitary-adrenal axis [8, 21]. CA can also stimulate ACTH release from pituitary cells in vitro [34]. In this equine study, plasma ACTH response during exercise was similar to that of both Ad and NA and there was a significant linear correlation between plasma ACTH and CA. Our data may support the assumption that possible ACTH releasing factors include circulating CA [21]. When the plasma CA and ACTH levels were related with HR response during incremental exercise, non-linear relationships were observed. Lehman et al. [18] reported that plasma CA abruptly increased at the aerobic-anaerobic transition range, which corresponded to a HR of about 175 beats/min in trained subjects. Plasma CA and ACTH abruptly increased at a HR over 200 beats/min in Thoroughbreds. The HR of 200 beats/min is regarded as an important index of aerobic power in the horse [24]. Accordingly, it is probable that plasma CA and ACTH responses to exercise abruptly change at the aerobic-anaerobic transition point. In addition, there were noticeable individual differences in plasma CA and ACTH responses in exercising Thoroughbreds. It is very interesting to know whether such individual differences are related to exercise performance or not, although we could not confirm the relationship in the present study.

In summary, the present data on plasma CA and ACTH responses to exhaustive incremental treadmill exercise suggest that the sympathoadrenal axis and the pituitary gland are highly activated by exercise in Thoroughbreds. The high activation of the sympathoadrenal axis and the pituitary gland in response to exercise may contribute to the superior athletic performance of Thoroughbreds. Plasma CA and ACTH levels during exercise were highly correlated with cardiorespiratory variables such as LA, HR and VO₂ and they abruptly increased around the aerobic-anaerobic transition point. These findings suggest that plasma CA and ACTH levels as well as LA and HR may be useful indicators for estimating exercise stress and physical fitness in Thoroughbreds.

Acknowledgments

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References

NEUROENDOCRINE RESPONSES TO TREADMILL EXERCISE OF THOROUGHBREDS


