



Study on Changes in Racehorses' Metabolites and Exercise-related Hormones before and after a Race

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ABSTRACT : Physiological changes in thoroughbred racehorses during the race were investigated by measuring concentrations of metabolites and exercise-related hormones before and after a race. The conversion point from anaerobic to aerobic exercise during the race was estimated subsequently. Blood samples were taken from the jugular vein of 53 thoroughbreds at different times -three h before and 45 min after- for measuring the concentrations of glucose, non-esterified fatty acids (NEFA), lactate, uric acid, ammonia, insulin, adrenocorticotrophin (ACTH) and cortisol according to the race distance. In accordance with the race distance, each metabolite increased in concentration compared with the level before the race. The level of glucose, in particular, increased from 56.18 ± 3.20 mg/dl before the race to 148.82 ± 8.82 mg/dl after the race for horses that raced 1,400 m, showing a significant increase of 165% ($p < 0.001$). The concentration of NEFA rose from 76.77 ± 5.59 μ Eq/L to 335.85 ± 35.39 μ Eq/L, up 337% ($p < 0.01$) after a 1,400 m race. Exercise-related hormones also showed similar changes. The level of insulin dropped the most in horses that raced 1,400 m, by 42%, from 0.97 ± 0.18 to 0.56 ± 0.05 μ g/L ($p < 0.5$); however, ACTH and cortisol jumped significantly at 1,800 m, from 20.17 ± 2.12 to 551.45 ± 91.33 pg/ml ($p < 0.5$) and 1.13 ± 0.16 to 5.66 ± 0.45 μ g/dl ($p < 0.01$), respectively, representing the highest increase. Therefore, based on the changes in glucose, NEFA and insulin levels before and after the race, it was concluded that the race distance of 1,400 m represents the point where racehorses make a conversion from anaerobic to aerobic exercise. (**Key Words :** Thoroughbred, Race, Race Distance, Metabolites, Hormones)

INTRODUCTION

Numerous studies have measured physiological changes in thoroughbreds in order to estimate their exercise capacity (Rose et al., 1990; Evans et al., 1993; Harkins et al., 1993; Ronéus et al., 1999; Inoue et al., 2002; Ju et al., 2002). Also, the results were determined to be useful in training racehorses (Davie and Evans, 2000). Glycogen and free fatty acids (FFA) play a key role when racehorses gallop, and changes in the levels of glucose, lactate, and FFA are important for racehorse trainers to know (Marion, 1975; Snow and Mackenzie, 1977). Plasma adrenocorticotrophin (ACTH) and cortisol are also key physiological markers when estimating the exercise capacity of racing horses

(Marc et al., 2000). Harkins et al. (1993) and Evans et al. (1993) studied the relationship between racehorses' racing capacity and physiological variables. Farrell et al. (1983) and Kraemer et al. (1989) reported the rise of ACTH levels in accordance with exercise intensity. The increased plasma ACTH due to the incremental intensifying of exercise causes plasma cortisol levels to rise (Farrell et al., 1983; Buono et al., 1986; Kraemer et al., 1989).

A few studies were conducted to measure the anaerobic and aerobic capacity of horses (Ronéus et al., 1994; Nummela and Rusko, 1995; Hinchcliff et al., 2002). Valberg et al. (1988) stated that a race includes maximal kinetic energy with maximal speed, and thus, requires both aerobic and anaerobic metabolism.

A traditional method of estimating the anaerobic capacity of men is to measure oxygen deficit levels (Volkov et al., 1975). Thus, Scott et al. (1991) measured oxygen deficit levels in horses, and Maxwell and Nimmo (1996) also conducted a study to understand the correlation between maximal anaerobic running and oxygen deficiency. As other ways to assess anaerobic capacity, Lacombe et al. (1999) and Saibene et al. (1985) each measured glycogen

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Table 1. Plasma glucose concentration in thoroughbreds before and after the race according to the race distance

Race distance (M)	N	Before the race (mg/dl)	After the race (mg/dl)	Percent increase
1,000	5	62.40±5.58 ¹	95.00±2.55 ^{d**}	52
1,200	8	54.00±4.39	111.25±9.41 ^{cd***}	106
1,400	11	56.18±3.20	148.82±8.82 ^{d***}	165
1,700	10	56.90±4.02	133.40±8.56 ^{abc***}	134
1,800	8	59.75±3.02	128.88±4.75 ^{abc***}	116
1,900	4	57.25±4.29	142.75±19.53 ^{ab*}	149
2,000	5	53.40±4.93	121.80±12.38 ^{bc**}	128

¹Mean±SE. ^{a-d} Values with different superscripts in the same column are significantly different ($p<0.05$).

P-value by paired t-test between before and after the race in the same row. *** $p<0.001$, ** $p<0.01$, * $p<0.05$.

Table 2. Plasma non-esterified fatty acid concentration in thoroughbreds before and after the race according to the race distance

Race distance (M)	N	Before the race (uEq/L)	After the race (uEq/L)	Percent increase
1,000	4	93.00±3.87 ^{1,a}	279.25±43.52 ^{abc*}	200
1,200	7	71.86±13.60 ^{ab}	237.14±17.89 ^{c**}	230
1,400	13	76.77±5.59 ^{ab}	335.85±35.39 ^{ab***}	337
1,700	10	96.70±16.79 ^a	351.40±28.37 ^{a***}	263
1,800	4	62.00±5.21 ^{ab}	214.00±16.83 ^{c**}	245
1,900	4	76.50±27.34 ^{ab}	294.75±50.31 ^{abc*}	285
2,000	4	50.75±13.62 ^b	242.50±14.29 ^{bc**}	379

¹Mean±SE. ^{a-c} Values with different superscripts in the same column are significantly different ($p<0.05$).

p-value by paired t-test between before and after the race in the same row. *** $p<0.001$, ** $p<0.01$, * $p<0.05$.

depletion and accumulation of lactate levels in blood.

Previous studies on metabolic hormones, however, were conducted using treadmills or race model experiments. Accurate research based on blood samples collected after an actual race has been not conducted yet.

Therefore, this study measured the concentration of metabolites and exercise-related hormones before and after a real race and suggested the conversion point from anaerobic exercise to aerobic exercise early in the race, estimating the physiological changes in accordance with the race distance.

MATERIALS AND METHODS

Horses

Fifty-three thoroughbreds were used in this study. 18 males, 27 females and 8 geldings. In terms of age, there were nine two-year-olds, 18 three-year-olds, six four-year-olds, seven five-year-olds, two six-year-olds, and one seven-year-old. The horses were under similar schedules everyday including warm-up training sessions at 05:30 and 14:00. They were fed three times a day at 09:00 h, 11:30 h and 16:00 h, with supplement feed at 21:00 h.

Races

The races were conducted with varying distances - 1,000, 1,200, 1,400, 1,700, 1,800, 1,900, 2,000 m - on an identical racetrack. The racetrack consisted of a 1,600 m-long inner track, a 1,800 m-long outer track, and a 900 m-long straightway

Blood sampling

Blood samples were collected from the jugular vein of horses at three h before and 45 min after the race by a veterinarian, and were stored in sodium-heparin vacutainer tubes. All blood samples were centrifuged to separate the plasma from the blood cells at 3,000 rpm, 4°C, 15 min, and the plasma was stored at -70°C until analysis.

Biochemical analysis

Glucose, non-esterified fatty acids (NEFA), and uric acid were analyzed with a blood automatic analyzer (Hiachi 7600-110/7170). Lactate and ammonia were analyzed with enzymatic colorimetrics (Cobas Integra 800). The sandwich linked immunoassay method using the measure kit (Mercodia, Bovine Insulin ELISA, Cat. No. 10-1131-01) was applied to measure insulin, and the radioactive immunoassay method (1470 Wizard- γ -counter) was used to measure adrenocorticotrophin (ACTH) and cortisol.

Statistics

We conducted the paired t-test procedure of the SAS (Statistical Analysis System) statistic package program (2001, release, 8.02 version) to verify the statistical significance of the changes in average concentration of each metabolite and hormone before and after the race.

RESULTS

Performance

The race times (average speed) of the racehorses

corresponding to each race distance were: 1:02 min (15.91 m/s) for 1,000 m, 1:17 min (15.53 m/s) for 1,200 m, 1:30 min (15.50 m/s) for 1,400 m, 1:54 min (14.85 m/s) for 1,700 m, 2:01 min (14.82 m/s) for 1,800 m, 2:07 min (14.86 m/s) for 1,900 m and 2:13 min (15.01 m/s) for 2,000 m.

Changes in blood parameters

Glucose : The level of glucose increased from 56.18 ± 3.20 mg/dl before the race to 148.82 ± 8.82 mg/dl at 1,400 m, showing a significant increase rate of 165% ($p < 0.001$). With the exception of 1,900 m, the increase levels decreased as the race distances extended beyond 1,400 m (Table 1).

NEFA : The concentration of NEFA rose from 76.77 ± 5.59 uEq/L to 335.85 ± 35.39 uEq/L, up 337% for 1,400 m, and maintained a high level as distance increased ($p < 0.001$, Table 2). The increase rate peaked at 378% after the 2,000 m race ($p < 0.01$).

Lactate : The concentration of lactate recorded the highest jump of 456% at 2,000 m ($p < 0.01$, Table 3). The

increase rates at 1,000 m and 1,800 m were 395% and 415%, respectively ($p < 0.001$).

Ammonia : Ammonia content rose after the race (Table 4). These ammonia concentrations even remained high for 60 min after the race. These levels did not show a pattern of consistent increase in relation to the race distance, but the increase rates at 1,000 m and 1,800 m were the same as 228% ($p < 0.001$).

Uric acid : Uric acid levels increased dramatically (Table 5). Except for the 1,900 m race distance, the average increase was over 1,000% ($p < 0.01$, $p < 0.001$). It is also worth noting that the increased levels of uric acids for distances below 1,400 m were 1.5 times higher than those over 1,400 m.

Insulin : The level of insulin dropped the most at 1,400 m, by 42%, from 0.97 ± 0.18 to 0.56 ± 0.05 $\mu\text{g/L}$ ($p < 0.5$, Table 6). In contrast, insulin levels in plasma samples increased at 1,700 m and 1,800 m.

ACTH : Concentration of ACTH in plasma samples showed a big change before and after the race, compared

Table 3. Plasma lactate concentration in thoroughbreds before and after the race according to the race distance

Race distance (M)	N	Before the race (mg/dl)	After the race (mg/dl)	Percent increase
1,000	5	$38.58 \pm 2.54^{1, ab}$	$190.98 \pm 11.18^{***}$	395
1,200	8	48.13 ± 3.03^a	$162.66 \pm 14.49^{***}$	238
1,400	13	46.05 ± 2.39^{ab}	$152.38 \pm 11.75^{***}$	231
1,700	10	43.01 ± 2.62^{ab}	$162.22 \pm 13.96^{***}$	277
1,800	8	37.41 ± 2.89^b	$192.61 \pm 12.76^{***}$	415
1,900	4	45.15 ± 4.06^{ab}	$159.63 \pm 19.68^{**}$	254
2,000	5	37.82 ± 3.99^b	$210.38 \pm 27.50^{**}$	456

¹ Mean \pm SE. ^{a-b} Values with different superscripts in the same column are significantly different ($p < 0.05$).

p-value by paired t-test between before and after the race in the same row. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.

Table 4. Plasma uric acid concentration in thoroughbreds before and after the race according to the race distance

Race distance (M)	N	Before the race (mg/L)	After the race (mg/L)	Percent increase
1,000	5	0.22 ± 0.04^1	$4.88 \pm 0.56^{***}$	2,118
1,200	8	0.26 ± 0.03	$4.85 \pm 0.58^{***}$	1,748
1,400	12	0.23 ± 0.03	$4.58 \pm 0.53^{***}$	1,933
1,700	10	0.29 ± 0.03	$4.79 \pm 0.44^{***}$	1,552
1,800	8	0.26 ± 0.03	$4.19 \pm 0.40^{***}$	1,495
1,900	4	0.33 ± 0.02	$3.33 \pm 0.64^*$	923
2,000	5	0.30 ± 0.00	$3.60 \pm 0.56^{**}$	1,100

¹ Mean \pm SE. p-value by paired t-test between before and after the race in the same row. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.

Table 5. Plasma ammonia concentration in thoroughbreds before and after the race according to the race distance

Race distance (M)	N	Before the race ($\mu\text{g/dl}$)	After the race ($\mu\text{g/dl}$)	Percent increase
1,000	5	$298.20 \pm 19.55^{1, c}$	$979.20 \pm 80.39^{abc***}$	228
1,200	8	323.88 ± 14.29^{bc}	$704.75 \pm 58.47^{d***}$	118
1,400	13	327.46 ± 14.64^{abc}	$808.77 \pm 66.79^{cd***}$	147
1,700	9	309.78 ± 18.36^{bc}	$874.44 \pm 73.50^{bcd***}$	182
1,800	8	317.25 ± 9.73^{bc}	$1,042.00 \pm 58.88^{ab***}$	228
1,900	4	373.75 ± 37.59^{ab}	$865.25 \pm 139.74^{bcd*}$	132
2,000	5	392.80 ± 21.44^a	$1,114.80 \pm 128.24^{a***}$	184

¹ Mean \pm SE. ^{a-d} Values with different superscripts in the same column are significantly different ($p < 0.05$).

p-value by paired t-test between before and after the race in the same row. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.

Table 6. Plasma insulin concentration in thoroughbreds before and after the race distance according to the race distance

Race distance (M)	N	Before the race ($\mu\text{g/L}$)	After the race ($\mu\text{g/L}$)	Percent increase
1,000	2	0.52 \pm 0.1 ¹	0.50 \pm 0.05	-2
1,200	5	0.39 \pm 0.05	0.52 \pm 0.07*	34
1,400	7	0.97 \pm 0.18	0.56 \pm 0.05*	-42
1,700	7	0.64 \pm 0.10	0.43 \pm 0.03	-33
1,800	4	0.45 \pm 0.04	0.43 \pm 0.03	-3

¹Mean \pm SE. p-value by paired t-test between before and after the race in the same row. * p<0.05.

Table 7. Plasma adrenocorticotrophin concentration in thoroughbreds before and after the race according to the race distance

Race distance (M)	N	Before the race (pg/ml)	After the race (pg/ml)	Percent increase
1,000	3	31.79 \pm 10.31 ¹	406.97 \pm 101.17 ^{abc}	1,180
1,200	4	21.80 \pm 9.66	220.39 \pm 62.04 ^c	572
1,400	3	16.02 \pm 0.44	362.66 \pm 58.63 ^{abc}	2,164
1,700	3	36.15 \pm 7.01	262.53 \pm 51.72 ^{bc}	626
1,800	3	20.17 \pm 2.12	551.45 \pm 91.33 ^{ab}	2,634
1,900	2	32.12 \pm 0.1	574.48 \pm 0.1	1,689
2,000	3	25.02 \pm 5.65	401.00 \pm 59.23 ^{abc} *	1,503

¹Mean \pm SE. ^{a-c} Values with different superscripts in the same column are significantly different (p<0.05).

p-value by paired t-test between before and after the race in the same row. * p<0.05.

Table 8. Plasma cortisol concentration in thoroughbreds before and after the race according to the race distance

Race distance (M)	N	Before the race ($\mu\text{g/dl}$)	After the race ($\mu\text{g/dl}$)	Percent increase
1,000	3	1.24 \pm 0.12 ¹	5.52 \pm 1.02	346
1,200	5	1.14 \pm 0.18	4.59 \pm 0.53**	302
1,400	3	1.73 \pm 0.38	5.83 \pm 0.72	237
1,700	3	1.59 \pm 0.52	5.78 \pm 0.65**	264
1,800	4	1.13 \pm 0.16	5.66 \pm 0.45**	402
1,900	3	1.42 \pm 0.16	4.84 \pm 0.24***	241
2,000	3	1.48 \pm 0.21	5.66 \pm 0.64*	282

¹Mean \pm SE. p-value by paired t-test between before and after the race in the same row. *** p<0.001, ** p<0.01, * p<0.05.

with the concentration of cortisol and insulin (Table 7). In 1,400 m, it rose from 16.02 \pm 0.44 to 362.66 \pm 58.63 pg/ml, which was not statistically significant but still remained high, and in 1,800 m, it jumped from 20.17 \pm 2.12 to 551.45 \pm 91.33 pg/ml, an increase of 2,634% (p<0.05). Also, the levels of ACTH for distances over 1,400 m were higher than those below 1,400 m.

Cortisol : Concentration of cortisol in plasma samples showed an opposite trend compared to ACTH, when divided into two different distance groups - below and over 1,400 m (Table 8). For 1,800 m, in particular, cortisol levels rose significantly by 402%, from 1.13 \pm 0.16 to 5.66 \pm 0.45 $\mu\text{g/dl}$ (p<0.01).

DISCUSSION

Performance

Average speed was recorded around 15 m/s until 1,400 m, and 14 m/s following 1,400 m, but it increased up to around 15 m/s again for 2,000 m. Factors affecting race time included a) individual differences among horses, b) the state of the race track, c) impost, as well as the race distance. Due to such complicated factors, there are limits to

measuring physiological changes of racehorses in absolute terms (Evans et al., 1993).

The increase pattern of glucose and NEFA

The glucose levels in both human and equine blood are affected by exercise intensity and exercise duration. Some studies have found that the conversion from trotting to galloping, which is equivalent to transferring from submaximal to maximal exercise, lowered the concentration of glucose (Engelhardt et al., 1973; Krzywanek, 1973; Lindholm and Saltin, 1974). While Snow and Mackenzie (1977) and Streter (1959) reported that glucose levels increased in blood samples taken from thoroughbreds after a race, Snow and Mackenzie (1977) also claimed that glycogen and FFA served as the source of kinetic energy for muscles and the concentration of the two substances increased after exercise. Glycogen and FFA are anaerobic substrates, which play a key role in the conversion from rest to the steady state exercise phase (Mark, 2000).

This study found that at around 1,400 m, the increase rate of glucose slowed and the concentration of plasma NEFA increased. According to the animal's physiology, when there is little supply of oxygen in the body the

primary response is to transform glycogen, which is stored in the liver and muscles, into glucose through glycogenolysis, resulting in the rise of glucose levels.

During aerobic exercise, the second reaction is to utilize fatty acids in adipocytes as additional supplies, enabling sustainable exercise. This study also shows that at around 1,400 m the increment of glucose and NEFA begins reversing course. In other words, the glucose level rises until 1,400 m and then starts falling above that distance, while the NEFA level dramatically increases beginning at 1,400 m. This might indicate that the 1,400 m distance is the threshold at which racehorses transfer from anaerobic exercise to aerobic exercise.

Changes in lactate

Lindholm and Saltin (1974) stated that racehorses' lactate content in blood and muscles increases dramatically at the maximum speed of 11.4-12.5 m/s. However, the average speed of horses in this study was higher than that, at 14.82-15.91 m/s, which explains why the lactate levels were higher than those in previous studies (Saibene et al., 1985; Ronéus, et al., 1994; Lacombe et al., 2001).

Generally, when an animal is given plenty of oxygen during exercise, pyruvate generated from glucose through glycolysis is transformed into acetate, and then oxidizes completely into H₂O, CO₂, and NADH after entering the citric acid cycle. In contrast, when there is a lack of oxygen, pyruvate is transformed into lactate, accumulating in blood and muscles and contributing to fatigue.

High-intensity cycle exercises conducted by men for 0.5-3 min accelerated the breakdown of glycogen, and this study found that 20-25% of the glycogen is transformed into glycolytic intermediates, 4-13% oxidized, and some 60% changed into lactate, including some in muscles (Medbo, 1993). In the case of horses, the increased level of lactate in the plasma after a treadmill exercise is the result of anaerobic glycolysis (Pösö et al., 1995). The increase of lactate and pyruvate concentration and lactate/pyruvate ratio in blood samples indicates that the muscles used much glucose (Marion, 1975).

This study shows that lactate levels increased slowly as race distances extended from 1,000 to 1,400 m. Until 1,400 m the lactate concentration increases are due to anaerobic exercise, but after passing the 1,400 m threshold the supply of oxygen in the body pulls down the heightened level of lactate. The fact that the lowest increment rate of 231% occurs at 1,400 m indicates a change in oxygen supply at that particular race length.

Changes in uric acid and ammonia

Even 60 min after the race, plasma uric acid and ammonia levels were still high. In the present study, there were previous results with similar findings in the blood

samples peaked at 40-60 min of recovery (Keenan et al., 1978; Harris et al., 1987; Ishida et al., 1999).

Sahlin et al. (1998) claimed that when ATP consumption exceeds its supply in a human body, the adenine nucleotide pool is partly deaminated, and this consequently forms IMP and ammonia. The byproducts of ATP breakdown are detectable in blood or muscles, and these can be used as a marker of energy deficiency. Similarly, in the case of horses, a single maximum gallop of 620 m and four 620 m gallops resulted in 1-26% and 16-51% drop of ATP in muscles, respectively, and the amount of decreased ATP was matched by an approximately equal rise in IMP. The IMP formed from AMP through AMP deaminase led to a rise in plasma uric acid levels (Snow et al., 1985).

Harris et al. (1987) stated that deamination of AMP will also result in the formation of ammonia, part of which will be taken up in the synthesis of glutamine and the rest released into the bloodstream. However, he claimed that uric acid detected in blood samples after an exercise is a better indicator of purine catabolism in muscles than ammonia. This claim was also supported in another study by Räsänen et al. (1996).

Changes in insulin

Numerous studies were conducted to find out the factors, which affect insulin resistance in mammals. There are at least two possible hypotheses: the increase of insulin could be due to hyperglycemia, and the drop in insulin could be due to the rise of FFA in plasma (Karen et al., 2006).

This study found that for a race distance of 1,400 m the concentration of insulin dropped significantly, by 42% (from 0.97±0.18 to 0.56±0.05 µg/L) while the levels of glucose and NEFA increased by 165% (from 56.18±3.20 to 148.82±8.82 mg/dl) and by 337% (from 76.77±5.59 to 335.85±35.39 uEq/L), respectively. Insulin levels increased by 34% after the 1,200 m race but they decreased after the 1,400 m race (by 42% for 1,400 m, 33% for 1,700 m, and by 3% for 1,800 m).

Two factors can explain the trend in insulin concentration. First, the rise in NEFA levels after the 1,400 m threshold pushes down insulin resistance. This falls in line with the observations made by Kjær et al. (1986, 1990) and Devlin et al. (1989), who in effect noted that for increased levels of catecholamin *in vivo*, FFA and glucose metabolites in cells obstructed glucose clearance by insulin after an exercise period. Second, insulin resistance is affected by the decrease of non-oxidative glucose disposal that is related to glycogen synthesis. The changes in insulin levels indicate that 1,400 m is the point at which metabolic substances are generated from anaerobic exercise, and subsequently accumulate in the blood stream and muscles. In other words, the 1,400 m threshold is where glycogen consumption is the highest in muscles, and where insulin

sensitivity is the lowest. In a study on insulin sensitivity changes related to completed exercise, Erik et al. (2001) claimed that insulin sensitivity is not correlated to the signal transduction system of the body but rather glycogen levels in muscles.

Changes in ACTH and cortisol

ACTH is a hormone secreted from the anterior pituitary, and it stimulates the adrenal cortex to secrete steroid hormones including cortisol (Farrell et al., 1983; Buono et al., 1986; Kraemer et al., 1989). ACTH levels increase dependency on exercise intensity (Farrell et al., 1983; Kraemer et al., 1989). Cortisol generated by the stimulus of ACTH transforms glycogen into glucose in the liver. It also activates gluconeogenesis and lipolysis, pushing up the concentration of glucose and FFA in the body (Irvine, 1983).

In this study, ACTH and cortisol both recorded the highest increment rates in the 1,800 m race (2.634% and 402%, respectively), rising to 27.34 times and 5.00 times, respectively. The rebound of glucose and NEFA levels at 1,800 m is related to the activation of gluconeogenesis and lipolysis, caused by the increment of plasma ACTH and cortisol contents at 1,800 m. The results were in line with a report by Masahiko et al. (1998), which found a substantial rise in ACTH and a small increase in cortisol after exercise, the amount of increases varied, however. In our study, ACTH increased by some 70 times, and cortisol by 1.8 times. As mentioned previously, variations in race environments and induced such gap differences.

CONCLUSION

Our results showed that at around 1,400 m, the elevated glucose levels started falling and NEFA levels increased dramatically. Insulin concentration, which is known to be related to glucose metabolism and fat metabolism, dropped more slowly from the 1,400 m mark, supporting the findings mentioned above. The 1,400 m track is composed of an 800 m-long straightway and a 600 m-long turn, which means receiving has less centrifugal force than other long race distance. In race distances shorter than 1,400 m, horses generated most of their energy through anaerobic metabolism; but from 1,400 m onward, they started aerobic exercises with the increased oxygen they inhale. However, it is important to remember that further research is needed as thoroughbred study results may vary according to individual differences in horses, the state of the race track, and impost as well as race distances.

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