Relationship between Serum Iron and Transferrin Concentrations in Light Breed Horses

Michinari YOKOHAMA, Kazushige MOGI
and Tatsuo Hosoda

Laboratory of Racing Chemistry, Setagaya-ku 158

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Abstract To clarify the physiological role of transferrin (Tf) in light breed horses, the relationship between serum iron and Tf concentrations was studied. Among the 15 Tf types, serum iron concentrations in animals with DF, DH, FF and FH types composed of D, F and H bands of fast running mobility and with a high level of Tf were observed between 81~86 $\mu\text{g/dl}$. On the other hand, those of animals with DR, FR, OR and RR types with a R band of slow running mobility and with a low level of Tf and also that of the DO type were found between 57~69 $\mu\text{g/dl}$. The difference between the two groups was significant ($P < 0.001$). Serum iron concentration in the Tf types having a silent gene was obviously lower than that of the normal Tf types. The differences of serum iron concentrations among six groups divided by distribution range of Tf concentration were significant ($P < 0.001$). Serum iron concentration in animals with a high level of Tf was also high. But, a tendency to have high ratio of serum iron to Tf concentration was recognized in animals with a low level of Tf. The developmental changes in serum iron concentration paralleled to those of the Tf concentrations. Throughout the age, serum iron concentrations in the Tf types having a silent gene were always 14~35 $\mu\text{g/dl}$ less than those of the normal Tf types and the difference of serum iron concentration was also obvious.

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As previously reported, the existence of a silent gene was newly observed in the equine serum Tf system¹⁾. Although it was difficult to distinguish Tf types having a silent gene from the normal Tf types with starch gel electrophoresis, it is possible to distinguish two types by the difference of their Tf concentrations²⁾. Factors which would have an influence on Tf concentration were studied to apply this technique to the blood typing in horses^{3,4,5)}. Accordingly, it is now imperative to study the physiological factors which will affect Tf concentration.

It has been well known that Tf is iron carrier-protein closely related with iron metabolism. Generally, it is assumed that, except during the time an individual is afflicted with a disease, each individual would contain the same value of serum iron for keeping one's metabolic function in normal condition. However, a recent report has been shown that there is a difference in the iron-binding capacity of Tf among the Tf types in cattle⁶⁾. If there are any relationships between Tf type and serum iron concentration, it will be possible to assess physiological significance of Tf concentration, since serum iron is closely related to synthesis of hemoglobin which is carrying the oxygen.

Serum Iron and Transferrin Protein Contents

The objective of this study is to clarify the relationship between serum iron and Tf concentrations with consideration of breed, Tf types and age.

Materials and Methods

Sample sera were collected from 281 horses consisting of 62 Anglo-Arabs and 219 Thoroughbreds. They were over 4 years of age. Studies on the developmental changes were carried out by using samples which are shown in Table 1. Blood samples were bled from the jugular vein by using the vaccum tube (TERUMO VENOJECT) from the population of breeding horses which we could not controll the condition of feed and management. A part of the results which previously reported⁴⁾ was used as Tf data in this experiment. The measurement of serum iron was determined using the TCA precipitation method by the International Committee for Standardization in Hematology⁷⁾. Tf concentration was measured by single radial immunodiffusion²⁾.

Table 1. Number of animals used in study on the developmental changes in serum iron concentration

Age	Normal transferrin type	Transferrin type having a silent gene
Hour after birth*		
1	0	2
Days of age		
1	0	2
2	0	2
3	0	1
4—7	5	2
8—11	17	1
12—15	7	2
16—19	16	0
20—26	20	1
30	20	0
Months of age		
2	20	2
3	20	1
4	20	1
5	19	1
6	20	1
7	20	1
8	16	1
Years of age		
2	20	4
3	0	2
Over 4	281	4

*: Before ingesting colostrum.

Results

1. *The relationship between serum iron and Tf concentrations*

Among the 15 Tf types, the FH type in the group (DF, DH, FF, FH and HH types) with a high level of Tf showed the highest value of serum iron (Table 2). On the other hand, the DR type in the group (DR, FR, HR, OR and RR types) with a low level of Tf was the lowest value of serum iron.

Tf types in which the difference was not significantly as compared with serum iron of the FH type were the DD, DF, DH, FF, HO and OO types. But, these six types did not completely belong to the group with a low level of Tf. On the other

Table 2. Relationship of serum iron to transferrin in transferrin types and breeds

Transferrin type	Anglo-Arab		Thoroughbred		Total	
	transferrin (mg/dl)	serum iron (μ g/dl)	transferrin (mg/dl)	serum iron (μ g/dl)	transferrin (mg/dl)	serum iron (μ g/dl)
DD	355.62 $\pm$ 7.51 (35)	97.50 (1)	349.59 $\pm$ 1.24 (253)	77.10 $\pm$ 3.03 (19)	350.32 $\pm$ 1.42 (288)	78.12 $\pm$ 3.05 (20)
DF	386.66 $\pm$ 11.34 (18)	98.50 (1)	383.80 $\pm$ 5.81 (36)	83.36 $\pm$ 4.14 (19)	384.75 $\pm$ 5.35 (54)	84.12 $\pm$ 4.00 (20)
DH	380.84 $\pm$ 9.96 (19)	82.79 $\pm$ 5.66 (8)	375.52 $\pm$ 9.30 (19)	83.71 $\pm$ 4.84 (12)	378.18 $\pm$ 6.73 (38)	83.34 $\pm$ 3.59 (20)
DO	351.44 $\pm$ 8.41 (11)	76.00 (1)	326.54 $\pm$ 5.54 (31)	65.95 $\pm$ 3.55 (19)	333.06 $\pm$ 4.90 (42)	66.46 $\pm$ 3.40 (20)
DR	316.20 $\pm$ 12.65 (16)	49.00 (1)	310.53 $\pm$ 8.09 (27)	57.72 $\pm$ 3.19 (19)	312.64 $\pm$ 6.82 (43)	57.28 $\pm$ 3.14 (20)
FF	396.65 $\pm$ 7.79 (43)		381.19 $\pm$ 2.89 (253)	81.29 $\pm$ 3.95 (20)	383.43 $\pm$ 2.73 (296)	81.29 $\pm$ 3.95 (20)
FH	379.58 $\pm$ 7.25 (32)	83.49 $\pm$ 4.66 (12)	372.84 $\pm$ 8.96 (20)	89.15 $\pm$ 4.43 (8)	376.98 $\pm$ 5.60 (52)	85.76 $\pm$ 3.29 (20)
FO	350.77 $\pm$ 10.41 (12)		329.86 $\pm$ 4.88 (30)	76.12 $\pm$ 3.19 (20)	335.83 $\pm$ 4.75 (42)	76.12 $\pm$ 3.19 (20)
FR	313.49 $\pm$ 8.51 (16)	89.70 $\pm$ 19.10 (2)	315.90 $\pm$ 6.66 (25)	67.24 $\pm$ 3.53 (18)	314.96 $\pm$ 5.18 (41)	69.49 $\pm$ 3.79 (20)
HH	380.50 $\pm$ 9.62 (23)	67.59 $\pm$ 5.25 (8)	351.74 $\pm$ 16.39 (5)	72.80 $\pm$ 0.70 (2)	375.33 $\pm$ 8.63 (28)	68.63 $\pm$ 4.20 (10)
HO	346.34 $\pm$ 14.26 (11)	87.80 $\pm$ 5.47 (10)	359.13 $\pm$ 13.51 (16)	72.69 $\pm$ 3.34 (10)	353.91 $\pm$ 9.79 (27)	80.25 $\pm$ 3.57 (20)
HR	319.52 $\pm$ 10.14 (22)	71.86 $\pm$ 3.09 (10)	344.15 $\pm$ 23.88 (13)	77.83 $\pm$ 4.49 (4)	328.67 $\pm$ 10.94 (35)	73.56 $\pm$ 2.57 (14)
OO	351.59 $\pm$ 16.14 (11)	92.40 $\pm$ 18.90 (2)	353.72 $\pm$ 5.03 (65)	79.27 $\pm$ 5.21 (18)	353.41 $\pm$ 4.85 (76)	80.59 $\pm$ 4.95 (20)
OR	313.51 $\pm$ 21.22 (6)	76.48 $\pm$ 7.74 (4)	314.57 $\pm$ 6.02 (24)	67.93 $\pm$ 4.29 (16)	314.36 $\pm$ 6.21 (30)	69.64 $\pm$ 3.76 (20)
RR	302.84 $\pm$ 10.47 (7)	80.15 $\pm$ 17.85 (2)	312.57 $\pm$ 8.78 (29)	67.17 $\pm$ 3.38 (15)	310.68 $\pm$ 7.33 (36)	68.69 $\pm$ 3.50 (17)
Mean	359.77 $\pm$ 3.15 (282)	79.89 $\pm$ 2.15 (62)	356.00 $\pm$ 1.51 (846)	73.88 $\pm$ 1.17 (219)	356.95 $\pm$ 1.38 (1128)	75.20 $\pm$ 1.04 (281)

(): Number of animals.

hand, the Tf type in which the difference was not significantly as compared with serum iron of the DR type was only the DO type, and the Tf types in which the difference was lower to some degree were the FR, HH, OR and RR types and these types were belonged to the group with a low level of Tf except for the HH type. The difference between the former and latter groups was significant as a result of variance analysis ($P < 0.001$). No difference of serum iron and Tf concentrations between the Thoroughbred and Anglo-Arab breeds was observed.

Ratio of animals having high value of serum iron became more and more higher as Tf concentration became high (Figure 1 and Table 3). Namely, when the distribution range of Tf concentration was between 250~300mg/dl, animals with serum iron concentration over 81 $\mu\text{g/dl}$ were only about 9%, and as Tf concentration increased to 300~350 mg/dl, 350~400 mg/dl and over 400 mg/dl, they were raised to 23%, 43% and 56%, respectively. Serum iron concentration of the Tf types having a silent gene was significant lower than that of normal Tf types. However, serum iron concentration of the former types was contained within the variable range of that of the latter types.

Fig. 1. Serum iron concentration in each range of transferrin concentration. *: Transferrin concentration in normal type. **: Transferrin concentration in type having a silent gene. ●: Mean.

Table 3. Serum iron concentration and rate of serum iron to transferrin in each range of transferrin concentration and transferrin types

Transferrin type	Distribution range of transferrin concentration (mg/dl)				
	241—250	250—300	300—350	350—400	Over 400
DD		67.60* 0.238** (1)	76.07±4.98 0.234 (10)	81.57±10.22 0.216 (9)	
DF			80.37±6.69 0.255 (3)	82.16± 6.31 0.216 (10)	88.51± 6.91 0.204 (7)
DH			56.90 0.182 (1)	83.24± 4.15 0.221 (15)	90.33± 5.64 0.214 (4)
DO		69.62±7.21 0.244 (5)	53.70±2.28 0.164 (4)	75.06± 4.73 0.203 (8)	55.23± 7.83 0.136 (3)
DR	64.37±0.67 0.261 (3)	51.53±2.70 0.179 (8)	58.20±7.70 0.182 (7)	66.45± 5.65 0.179 (2)	
FF			71.68±1.24 0.221 (4)	89.85± 7.37 0.240 (10)	72.08± 4.94 0.172 (6)
FH			89.20 0.273 (1)	82.81± 4.31 0.220 (13)	91.57± 5.45 0.217 (6)
FO			69.87±5.20 0.214 (7)	77.11± 4.35 0.202 (10)	87.40± 7.82 0.195 (3)
FR		64.53±6.93 0.223 (3)	71.30±5.36 0.225 (11)	63.63± 7.91 0.178 (6)	
HH		68.60 0.254 (1)	61.55±5.65 0.185 (2)	64.95± 8.78 0.174 (4)	78.27± 5.28 0.181 (3)
HO			78.14±5.62 0.236 (10)	81.30± 6.23 0.213 (7)	84.80± 6.42 0.205 (3)
HR		74.02±5.14 0.272 (5)	75.67±8.01 0.233 (3)	69.90± 2.36 0.194 (5)	83.30 0.199 (1)
OO		73.50 0.250 (1)	69.23±4.34 0.211 (12)	112.75±11.25 0.295 (2)	96.38± 9.17 0.224 (5)
OR		60.55±2.10 0.211 (4)	64.14±3.89 0.198 (7)	74.51± 4.64 0.196 (7)	89.95±33.51 0.222 (2)
RR	62.93±7.08 0.254 (3)	52.60±2.56 0.187 (4)	76.84±3.99 0.237 (9)	77.00 0.216 (1)	
Mean	63.62±3.19 0.257 (6)	62.07±2.22 0.219 (32)	70.69±1.40 0.218 (91)	79.71± 1.67 0.212 (109)	84.30± 2.73 0.198 (43)

*: Serum iron concentration ($\mu\text{g/dl}$), mean±S.E., **: Rate of serum iron to transferrin ($\times 10^{-3}$). (): Number of animals.

With the exception of the DO, FF, FR and HR types, the following tendency was recognized: serum iron level became high as the Tf concentration increased. However, the ratio of serum iron to Tf concentration decreased as Tf concentration increased. And then the difference of serum iron concentration among Tf types was significant ($P<0.001$).

2. Developmental changes in serum iron concentration

The developmental changes in serum iron concentration of normal Tf types and that of Tf types having a silent gene were presented in Figure 2. Serum iron concentration in normal Tf types was somewhat low at 4~7 days of age. The highest

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Fig. 2. Developmental changes in serum iron and rate of serum iron to transferrin.

—●—● Serum iron
 —○—○ Rate of serum iron to Tf
 —●—● Serum iron
 —○—○ Rate of serum iron to Tf

: Mean $\pm$ S. E. Normal Tf types
 — Tf type having a silent gene

peak of the value was found at 20~26 days of age, and thereafter, it decreased without any remarkable variation and became almost a constant value from 3 months to 2 years of age. Then, it decreased gradually to 75 $\mu\text{g/dl}$ for horses over 4 years of age. On the other hand, in Tf types having a silent gene, serum iron concentration at one hour after birth was remarkably high. Thereafter, it decreased remarkably and reached the lowest value at 4~7 days of age. It increased again from 8~11 days of age, and was 78 $\mu\text{g/dl}$ at 2 months of age. Serum iron concentration decreased without any remarkable variation to 63 $\mu\text{g/dl}$ at 2 years of age and 57 $\mu\text{g/dl}$ for the horses over 3~4 years of age.

In those over 4~7 days of age, serum iron concentration of the normal Tf types showed a higher level than those of the Tf types having a silent gene and a distinct difference was recognized between the two types. However, the ratio of serum iron to Tf concentration in Tf types having a silent gene was higher than that of the normal Tf types.

Discussion

As has been previously reported on the difference of Tf concentration among Tf types, the DF, DH, FF, FH and HH types consist of a group with a high level of Tf, whereas, the DR, FR, HR, OR and RR types make up another group with a low level of Tf^{4,5)}. The difference of serum iron concentration between these two groups was

observed just as found in their Tf concentrations. Although Tf concentration in the HH type belonged to the group with a high level of Tf, the value of its serum iron was not contained in the group with the high level of serum iron. Serum iron concentration of the FH type was higher than those of the DR type and Tf types having a silent gene. Although it has not been clarified whether this difference of serum iron concentration in horses will have any physiological effects on iron metabolism, it may be a better condition for individual life that both of the Tf and serum iron concentrations are a high level.

As a general rule, fluctuations of serum iron is influenced by environmental factors, and especially, the diurnal variation is remarkable. Obara *et al.* (1957)⁸⁾ has been reported that fluctuations of serum iron level during a day and a week were in range of $\pm 25 \mu\text{g/dl}$ in horses. Since blood samples used in this study were collected from the equine population that we could not control on the conditions of feed and management, the fluctuations of serum iron might be remarkable. However, as a large number of animals were used for this study, the variations would be eliminated.

From the studies on the relationship between serum iron and Tf concentrations in the normal Tf types, it was clarified that the frequency of the appearance of animals having a high level of serum iron increased as Tf concentration became higher. The average value of serum iron in Tf types having a silent gene was obviously lower than that of the normal Tf types. But, the value was within the normal range. Accordingly, when serum iron concentration is used as an index for distinguishing Tf types having a silent gene from normal Tf types, it is less useful than Tf concentrations.

It was also clarified from the results concerning the ratio of serum iron to Tf concentration that the variation of serum iron in Tf types having a silent gene was contained within that of the normal Tf types. The ratio of serum iron to Tf concentration became high in animals with low level of Tf, and thus, it seems likely that this phenomenon was induced by physiological response for keeping a certain serum iron level.

The developmental changes in serum iron concentration paralleled to those of the Tf concentration. Namely, as has been already reported concerning the developmental changes of Tf concentration^{8,9)}, serum iron concentration were also higher in foals than that of adult horses.

It is presumed that animals with a low level of Tf have the physiological function for increasing serum iron to a high value, and in reverse, unsaturated iron-binding capacity of Tf decrease in these animals. Since it has been reported that Tf has a sterilizing effect on bacteria and virus^{10,11)}, the susceptibility to infection may be increased in horses with a low Tf concentration.

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馬の血清 transferrin 蛋白量と血清鉄値との関係

横浜道成・茂木一重・細田達雄

競走馬理化学研究所, 世田谷区 158

馬の血清 transferrin (Tf) 蛋白量の生理学的役割について考察するため, 品種, Tf 型および年齢の各要因から見た Tf 蛋白量と血清鉄値との関連性について調べた。1. 15 種類の Tf 型のうち, 易動度の早い D, F および H バンドから成る DF 型, DH 型, FF 型および FH 型の血清鉄値は 81~86 $\mu\text{g}/\text{dl}$ の間にあり, また, 主に易動度の遅い R バンドを含む DR 型, FR 型, OR 型および RR 型並びに DO 型の血清鉄値は 57~69 $\mu\text{g}/\text{dl}$ の間にあり, この両群間には有意の差が認められた ($P < 0.001$)。2. Silent gene をもつ Tf 型の個体の平均血清鉄値 (57 $\mu\text{g}/\text{dl}$) は通常の Tf 型の個体の平均値 (75 $\mu\text{g}/\text{dl}$) よりも明らかに低い値であったが, その変動幅は通常の Tf 型の個体の

変動幅の範囲内であった。3. Tf 蛋白量を 6 段階に分けて (図 1), その血清鉄値の差を分散分析した結果, 有意な差が認められた ($P < 0.001$)。また, Tf 蛋白量の高い個体は血清鉄値も高い値を示した。しかし, Tf 蛋白量が少ない個体は Tf に対する血清鉄の比率が高くなる傾向を示した。4. 血清鉄値の年齢的変動は Tf 蛋白量の変動推移と平行しており, また, 各年齢において, silent gene をもつ Tf 型は通常の Tf 型に比べ血清鉄値が 14~35 $\mu\text{g}/\text{dl}$ 少なく, 明白な差が認められた。以上の如く, Tf 蛋白量が高い個体は血清鉄値も高い傾向を認めたが, これの鉄代謝にもつ意義は不明である。

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