

# Serum lipid and lipoprotein patterns of Iranian horses

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## Summary

Patterns of serum biochemical parameters vary among horse breeds. The objective of the present study was to compare serum lipoproteins of Iranian Caspian ponies with those of other horses (Arabs and Thoroughbreds) in the Iranian region. Serum lipoprotein values were determined by agar-agarose gel electrophoresis and measured by scan densitometry. Moreover, serum triglyceride and cholesterol concentrations were determined and the results were analysed by one-way analysis of variance. Serum triglyceride and cholesterol values were  $1.13 \pm 0.23$  and  $2.38 \pm 0.18$  mmol/l in Caspian ponies,  $1.96 \pm 0.49$  and  $1.92 \pm 0.25$  mmol/l in Arab horses and  $1.38 \pm 0.26$  and  $2.17 \pm 0.53$  mmol/l in Thoroughbred horses. The relative percentages of  $\alpha$ - (72.63  $\pm$  17.76%) and  $\beta$ -lipoproteins (29.10  $\pm$  5.49%) in serum electrophoretic tracings from Caspian ponies were not significantly different from those of other horses ( $p > 0.05$ ). The lipoprotein phenotype in Caspian ponies may be useful for evaluating metabolic diseases.

## Keywords

Horse – Iran – Iranian Caspian pony – Lipid – Lipoprotein – Serum.

## Introduction

The Caspian pony is a wild animal that lives in wet climate conditions and is native to the mountainous areas in the north of Iran. The breed has a unique adaptation to humid environments. Researchers believe that Iranian Caspian ponies are actually a breed of small horse rather than a type of pony, and because they live in south-eastern areas of the Caspian Basin they are also called Caspian miniature horses (1). These animals are extremely rare and were pulled back from the brink of extinction in 1965 (2).

Breed variations within domestic and wild animal species mean that determination of the serum lipoprotein profile is of interest in many clinical applications. Studies on serum

lipid transport systems have shown significant differences among different animals, and several studies have evaluated serum lipid and lipoprotein parameters in horses (3, 4, 6, 7). Differences in lipid profiles between ponies and horses are evident after fasting, when the serum of ponies is visibly lipaemic and that of horses is not (8). Metabolic disorders are prevalent in both horses and ponies, and ponies in particular are at risk of laminitis as a consequence of disorder in the metabolism of lipids and lipoproteins. In this regard, it has been suggested that laminitis may be triggered by mild hypertriglyceridaemia in ponies (9).

Equine metabolic syndrome is a term that refers to a collection of clinical signs that contribute to the development of laminitis in horses. This syndrome is

defined by insulin resistance, obesity and/or regional adiposity, with prior or current laminitis (5). Hyperlipaemia arises as a secondary complication of a primary systemic disease such as laminitis in the majority of cases in miniature horses (10). Measurement of serum triglyceride (TG) concentrations appears to be the best method of diagnosing hyperlipaemia. High levels of serum cholesterol are another diagnostic marker for hyperlipaemia in horses (11).

It appears that lipid and lipoprotein parameters show different patterns among different breeds of horse. Some categorise different equine breeds into easy-to-maintain and hard-to-maintain breeds. Researchers believe that easy-to-maintain breeds (such as most ponies) and hard-to-maintain breeds (such as Thoroughbreds) differ in their lipid and lipoprotein status (8). Like lipid profiles, lipoprotein profiles may be used to detect and classify diseases of lipid metabolism. Furthermore, equine lipoproteins have been compared with those of other species and contrasted between healthy and diseased horses. In this respect, variations exist in lipoprotein profiles within equine species (4). Given that no studies have addressed the lipoprotein profiles of Iranian Caspian ponies, the aim of the present study was to determine the serum lipoprotein pattern of the Iranian Caspian pony and to compare the values with those from Arab and Thoroughbred horses.

## Materials and methods

In this study, 24 Thoroughbred horses (15 females and 9 males), 16 Arab horses (5 females and 11 males) and 11 Iranian Caspian ponies (2 females and 9 males) were studied. The Thoroughbred and Arab horses were kept at the Chogan Institute (an equestrian studies institute in Karaj, Tehran, Iran) and the Iranian Caspian ponies were kept at the related horse-training centre of Shahid Zamanpour (Karaj, Tehran, Iran). Samples were collected in mid-July 2008 after the owners of all the horses had completed a consent form. The horses were kept in individual box stalls at night and were released each day into a dirt pen. The ration provided was a commercially pelleted horse feed (Pars Animal Feed Company, Tehran, Iran) consisting of approximately 12.5% protein, 4% fat, 12% fibre, 52% nitrogen-free extract, 12% moisture and 7.5% ash. The amount of the ration given to each animal was determined on the basis of body weight (1.5 kg pellets/100 kg of body weight). The horses were fed half of this amount twice a day, in the morning and in the afternoon. At noon the horses were given approximately 1 kg of alfalfa and approximately 1 kg of hay. Feed was withheld from the horses and ponies approximately 16 h before blood was collected, but free access to water was permitted. Blood samples (10 ml) were

collected by jugular venepuncture into vacutainer tubes (Pars-Khavar, Alborz Industrial Town, Tehran, Iran). After about 30 min, the samples were centrifuged at 1,000 g for 15 min in a table-top centrifuge. Serum was separated immediately, aliquoted into cryotubes (Nunc Brand Products, Nalge Nunc International, Roskilde, Denmark) and stored for no longer than one month in a freezer at  $-80^{\circ}\text{C}$  while awaiting analysis. Serum samples were analysed for TG concentration by the glycerol-phosphate oxidase *p*-amino phenazone method and for total cholesterol (TC) concentration by the cholesterol oxidase *p*-aminophenazone method using an ultraviolet (UV)/visible spectrophotometer (UV-120-12, Shimadzu Corp., Kyoto, Japan) and reagents obtained from the Zist Chimi Chemical Company (Tehran, Iran).

Serum lipoprotein electrophoresis was performed according to Noble's method, with some modifications (14). Briefly, agarose gel was prepared by mixing 0.05% agarose, 0.6% agar and 2.5 g/l bovine serum albumin (BSA, Merck Supplier Co., Tehran 15178, Iran) (4:1; 0.1; v/v/v). Agarose and agar were obtained from Roche Diagnostics (GmbH, Toba Negin, Tehran, Iran). Electrophoresis was performed at 120 V and  $10^{\circ}\text{C}$  for 2.5 h. The gels were fixed in 50% methanol and dried at  $60^{\circ}\text{C}$  overnight. The gels were stained using Sudan black stain (Sudan black 0.4 g; ethanol 120 ml; zinc acetate 4 g;  $\text{dH}_2\text{O}$  80 ml) (Merck Supplier Co., Tehran, Iran) for 2 h and destained using tap water. The gels were scanned using a lipoprotein power scan densitometer (Nima Pooyesh Teb Company, Tehran, Iran). Statistical analysis was performed by one-way analysis of variance (ANOVA) among breeds using Sigma Stat 2 (Systat Software Inc., Point Richmond, CA, USA). Data were tested for normality;  $\alpha$  in all cases was 5% (i.e.  $p < 0.05$ ).

## Results

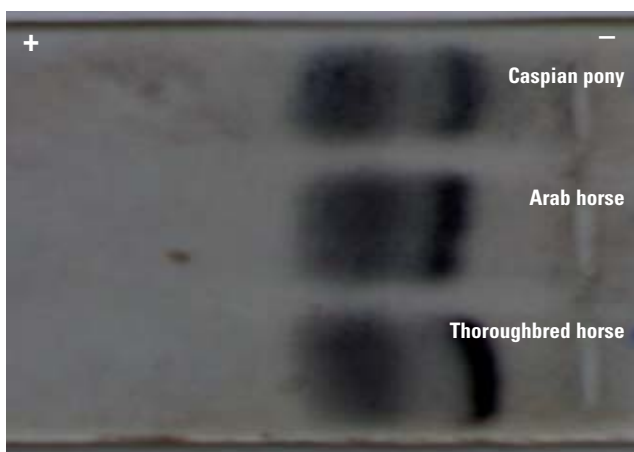
The serum TG and TC concentrations in all three breeds were tabulated (Table I). Statistical analysis revealed no significant difference between Iranian Caspian ponies and Thoroughbreds in terms of TG and TC. On the other hand, while serum TG values in the Arab horses were higher than in both Iranian Caspian ponies and Thoroughbred horses ( $p < 0.001$ ), their serum TC values were lower than those of the Iranian Caspian ponies ( $p = 0.015$ ).

Agar-agarose gel lipoprotein electrophoresis showed two major serum lipoprotein fractions in all the horses (Fig. 1). Figures 2a, 2b and 2c show typically larger percentages for  $\alpha$ -lipoproteins when compared with  $\beta$ -lipoproteins in scan densitometric peaks of the lipoprotein bands. The relative percentages of  $\alpha$ - and  $\beta$ -lipoproteins are shown in Table II. Statistical analysis showed no significant differences in lipoprotein profiles among the breeds ( $p > 0.05$ ).

**Table I**  
**Equine serum triglyceride and total cholesterol concentrations**  
 Values are expressed as mean ± standard deviation

Breed	Number tested	Triglyceride (mmol/l)	Total cholesterol (mmol/l)
Thoroughbred	24	1.38 ± 0.26 <sup>(a)</sup>	2.17 ± 0.53
Arab	14	1.96 ± 0.49 <sup>(a, b)</sup>	1.92 ± 0.25 <sup>(c)</sup>
Iranian Caspian pony	12	1.13 ± 0.23 <sup>(b)</sup>	2.38 ± 0.18 <sup>(c)</sup>

In each row, identical letters show significant differences <sup>(a), (b)</sup>  $p < 0.001$ ; <sup>(c)</sup>  $p = 0.015$



-: Anode      +: Cathode

**Fig. 1**  
**Serum agar-agarose gel electrophoresis of serum lipoproteins in Iranian Caspian ponies, Thoroughbred horses and Arab horses**

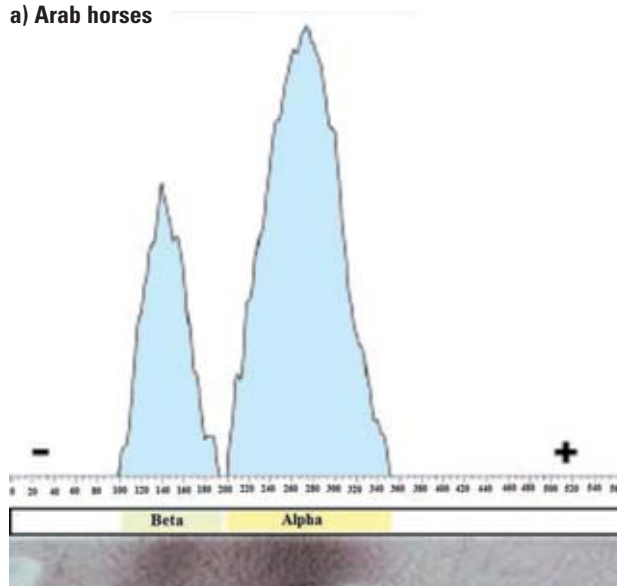
**Table II**  
**Equine serum lipoproteins separated on agar-agarose gel electrophoresis**  
 Values of band relative percentages are expressed as mean ± standard deviation. There were no statistically significant differences in serum lipoproteins ( $p > 0.05$ )

Breed	Number tested	α-lipoproteins (%)	β-lipoproteins (%)	α/β (%)
Thoroughbred	24	68.63 ± 8.71	31.56 ± 7.44	0.47 ± 0.14
Arab	14	67.25 ± 8.01	32.90 ± 7.74	0.51 ± 0.19
Iranian Caspian pony	12	72.63 ± 17.76	29.10 ± 5.49	0.41 ± 0.12

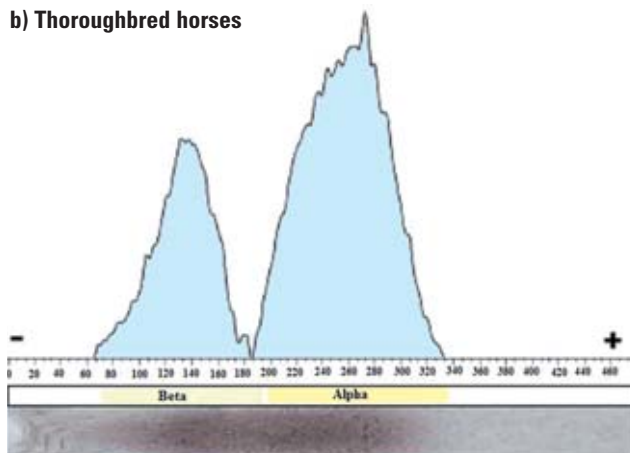
## Discussion

In this study, serum TG concentrations in Iranian Caspian ponies ( $1.13 \pm 0.23$  mmol/l) were higher than those reported in the Nonius horse ( $0.7$  mmol/l) (18) and the Shetland pony ( $0.8 \pm 0.11$  mmol/l) (15), but were similar

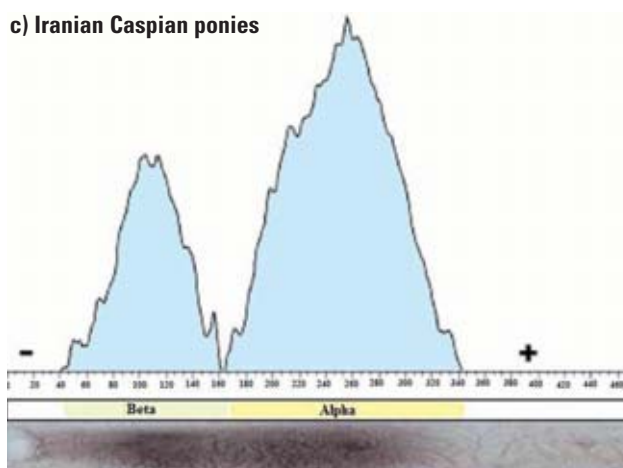
**a) Arab horses**



**b) Thoroughbred horses**



**c) Iranian Caspian ponies**



-: Anode      +: Cathode

**Fig. 2**  
**Densitometric peaks indicate the β-lipoprotein (left peak) and α-lipoprotein (right peak) regions in Arab horses, Thoroughbred horses and Iranian Caspian ponies**

to those reported in the Morgan horse ( $1.06 \pm 0.12$  mmol/l) (16). In the present study, serum TG values in Iranian Caspian ponies were similar to those of Iranian Thoroughbreds and lower than those of Arab horses. However, serum TG values were higher than those reported previously in Iranian Caspian ponies ( $0.14\text{--}0.28$  mmol/l) (13). The serum TG levels in Thoroughbred horses were also higher than values reported previously in Thoroughbreds ( $0.26 \pm 0.16$  mmol/l) (16) and in Arabs ( $0.69 \pm 0.16$  mmol/l) (9). With regard to these differences, the literature states that blood parameters in horses are not the same as those of the dog or cat. Biological factors (e.g. breed variations, strain within a breed, oestrus, sex, time of day, elevation) and non-biological reasons (e.g. technical error, sampling error, laboratory error) could potentially cause the differences in TG among the horse breeds in this study (8, 12).

The mean serum TC concentrations in Iranian Caspian ponies were higher than those reported in Nonius horses ( $1.86\text{--}1.89$  mmol/l) (18), but lower than those in Morgan horses ( $3.6 \pm 0.14$  mmol/l) (16). Serum TC values in Iranian Caspian ponies were similar to those reported in the Thoroughbred ( $2.92 \pm 0.21$  mmol/l) (15), Shetland pony ( $2 \pm 0.4$  mmol/l) (2) and Cheval de Selle ( $2.34$  mmol/l) (3). However, the values obtained in the present study were lower than those reported previously in the Iranian Caspian pony ( $3.09\text{--}5.07$  mmol/l) (13). The present study did not show any difference in the serum TC values between the Thoroughbred and the Iranian Caspian pony or between Thoroughbred and Arab horses.

In the present study, as in previous studies of Morgan and Thoroughbred horses (17), agar-agarose gel lipoprotein electrophoresis demonstrated two major lipoprotein regions in Iranian Caspian ponies. The mean percentage of  $\alpha$ -lipoproteins in Iranian Caspian ponies was similar to those found in Thoroughbred and Arab horses (17).

In conclusion, the results of this study defined the serum TG and TC concentrations and the lipoprotein profile in the Iranian Caspian pony. These data might be useful for further studies evaluating differences in lipoproteins among animal species and for further evaluation of lipid and lipoprotein profiles in ponies with systemic diseases, such as laminitis.

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## Structure des lipides et des lipoprotéines sériques chez des équidés iraniens

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### Résumé

Chez les équidés, la structure des paramètres biochimiques du sérum varie d'une race à l'autre. Les auteurs présentent les résultats d'une étude visant à comparer les concentrations sériques des lipoprotéines chez des poneys caspiens d'Iran par rapport à d'autres races équinées (arabe et pur-sang) présentes en Iran. Les valeurs des lipoprotéines sériques ont été mesurées par électrophorèse sur gel d'agarose suivie d'un tracé densitométrique. En outre, il a été procédé à un relevé des concentrations des triglycérides et du cholestérol, lequel a été soumis à une analyse de la variance à un facteur. Les valeurs des triglycérides et du cholestérol étaient, chez les poneys caspiens, respectivement de  $1,13 \pm 0,23$  et de  $2,38 \pm 0,18$  mmol/l ; chez les chevaux de race arabe, de  $1,96 \pm 0,49$  et de  $1,92 \pm 0,25$  mmol/l ; chez les chevaux de race pur-sang, de  $1,38 \pm 0,26$  et de  $2,17 \pm 0,53$  mmol/l. Les taux relatifs des lipoprotéines  $\alpha$  ( $72,63 \pm 17,76$  %) et  $\beta$  ( $29,10 \pm 5,49$  %) relevés dans les tracés électrophorétiques du sérum des poneys caspiens ne présentaient pas de variation significative ( $p > 0,05$ ) par rapport aux autres races étudiées. Le phénotype des lipoprotéines chez les poneys caspiens constitue un paramètre intéressant pour évaluer les maladies métaboliques.

### Mots-clés

Cheval – Iran – Lipide – Lipoprotéine – Poney caspien d'Iran – Sérum.



## Distribución sérica de lípidos y lipoproteínas en caballos iraníes

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### Resumen

Los parámetros bioquímicos del suero difieren entre las distintas razas de caballo. Los autores describen un estudio destinado a comparar las lipoproteínas séricas de ponis del Caspio iraníes con las de otras razas (árabes y purasangre) de la región iraní. Por electroforesis en gel de agarosa se determinaron las lipoproteínas presentes en el suero, que después se cuantificaron por densitometría de barrido. Además, se midieron las concentraciones séricas de triglicéridos y colesterol, y se sometieron los resultados a un análisis de la varianza unifactorial. Se obtuvieron los siguientes valores de triglicéridos y colesterol en suero:  $1,13 \pm 0,23$  y  $2,38 \pm 0,18$  mmol/l en ponis del Caspio;  $1,96 \pm 0,49$  y  $1,92 \pm 0,25$  mmol/l en caballos árabes; y  $1,38 \pm 0,26$  y  $2,17 \pm 0,53$  mmol/l en los purasangre. El porcentaje relativo de  $\alpha$ - ( $72,63 \pm 17,76\%$ ) y  $\beta$ -lipoproteínas ( $29,10 \pm 5,49\%$ ) en los perfiles electroforéticos del suero de los ponis del Caspio no resultó significativamente distinto del de los demás caballos ( $p > 0,05$ ). El fenotipo de lipoproteínas de los ponis del Caspio puede ser útil para evaluar enfermedades metabólicas.

### Palabras clave

Caballo – Irán – Lípido – Lipoproteína – Poni del Caspio iraní – Suero.



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