

Serum lipids and lipoproteins in clinically healthy Caspian miniature horses

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ABSTRACT

Blood samples were collected from the jugular vein of 50 clinically healthy Caspian miniature horses according to their age (1.5-3, 3-5 and >5 years) and sex. Variations in the serum concentrations of cholesterol, triglycerides, total lipid, very low-density lipoprotein, (VLDL-cholesterol), low-density lipoprotein (LDL-cholesterol) and high-density lipoprotein (HDL-cholesterol) were investigated. The concentrations of cholesterol, triglycerides, total lipid, HDL-cholesterol, LDL-cholesterol and VLDL-cholesterol in the different age groups were significantly different ($P<0.05$). With an increase in the age of animals there were significant increases ($P<0.05$) in cholesterol, triglycerides, total lipid, HDL-cholesterol, LDL-cholesterol and VLDL-cholesterol concentrations. However, sex showed no significant differences on the concentrations of these parameters.

Key words: lipid, lipoprotein, serum, Caspian miniature horse

Introduction

Studies on cholesterol, triglycerides and lipoproteins in domestic animals have clearly shown that species variations exist and that even within species significant differences occur. The normal concentrations of serum lipids and lipoproteins of the horse and pony in various physiological conditions have been reported (ROBERTZ, 1974; BAUER, 1983; SAKO et al., 1989; KANEKO, 1989; WASTON et al., 1993a; WASTON et al., 1993b; DUNCAN et al., 1994). Perhaps the most exciting equine discovery of the 20th century was that the

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Caspian is not a pony but an ancient breed of miniature horse, previously believed to have been extinct for over a thousand years. This tiny horse was probably one direct ancestor of the oriental breeds, and subsequently of all light horse breeds. These animals are extremely rare and were pulled back from the brink of extinction in 1965 by Louise Firouze, an American living in Iran (HENDRICKS, 1995). The Caspian miniature horse is a native of the area around the Elborz Mountains and Caspian Sea in Iran. The current theory is that the Caspian is the ancient miniature horse of Mesopotamia which, after being used by the Mesopotamians in the third millennium before Christ until the seventh century AD, was believed to have become extinct (EDWARDS, 1985). Establishing continuity for a breed for more than 3000 years will be difficult, but failure to take an exhaustive look into the past of this unique little horse may result in the loss of the vestige of the wild horse of the Middle East. The greatest importance of the Caspian lies in the fact that if continuity can be proven, the breed is likely to be the ancestor of all modern breeds of hot-blooded horses (EDWARDS, 1992; HENDRICKS, 1995; DRAPER, 1996).

There is no published information about the lipid profile of the Caspian miniature horse. The present study was therefore conducted to estimate the normal values of serum lipids (cholesterol, triglycerides, total lipid) and lipoproteins (HDL-cholesterol, LDL-cholesterol and VLDL-cholesterol) in clinically healthy Caspian miniature horses.

Materials and methods

Blood samples were collected from 50 adult Caspian miniature horses according to their age (1.5-3, 3-5 and >5 years) and sex. The horses had been reared in the province of Golestan, north-eastern Iran. Animals were kept in the range pasture of an area near the Caspian Sea and Elborz Mountains. The climate is temperate with high humidity. The predominant pasture plants consisted of a mixture of legume and grasses where animals were fed only on the pasture with free access to water. No feed supplements were offered. All the animals were clinically healthy and free from internal and external parasites. Each horse has a separate file including all necessary records, and the age of the horses was determined by referring to these records. Blood samples were taken from the jugular vein into vacuum containers (Becton Dickinson Co., New Jersey, U.S.A.) and the serum was separated following centrifugation for 15 min at 750×g. Any hemolysed samples were discarded. Serum samples were stored at -20 °C until analysed for cholesterol using a modified Abell-Kendall/Levey-Brodie (A-K) method (BURTIS and ASHWOOD, 1994), for triglycerides using the enzyme procedure of McGOWAN et al. (1983) and for total lipid using the method described by ZOLLNER and KIRSCH (1962). Lipoproteins were isolated using a combination of precipitation and ultracentrifugation. High density lipoprotein cholesterol was measured by a precipitation method. In the first step, the precipitation reagent (sodium phosphotungstate with magnesium chloride) was added to serum to aggregate non-HDL

lipoproteins, which were sedimented by centrifugation ($10000 \times g$ for 5 min). The residual cholesterol was then measured using an enzymatic method (BURTIS and ASHWOOD, 1994). Cholesterol was quantified in the serum precipitate and in the high density lipoprotein supernatant using the enzymatic method. Low density lipoprotein cholesterol was calculated as the difference between cholesterol measured in the precipitate and in the high density lipoprotein fraction. Very low density lipoprotein cholesterol was estimated as one-fifth of the concentration of triglycerides (FRIEDEWALD et al., 1972).

The data were expressed in SI units and analysed by one-way ANOVA and regression analysis using SPSS/PC software, and Duncan's multiple range test was used to detect significant differences between means (NORUSIS, 1993). All values were expressed as mean and standard error (SEM), with $P < 0.05$ indicating statistical significance.

Results

The mean \pm standard error of serum lipids and lipoproteins of adult Caspian miniature horses in different age groups and both sexes are presented in Tables 1 and 2, respectively. It will be seen that age had a significant effect on the serum lipid and lipoprotein concentrations of the Caspian miniature horses, as with an increase in the age of animals there was an increase in the cholesterol ($P < 0.05$; $r = 0.89$), triglycerides ($P < 0.05$; $r = 0.47$), total lipid ($P < 0.05$; $r = 0.61$), HDL-cholesterol ($P < 0.05$; $r = 0.60$), LDL-cholesterol ($P < 0.05$; $r = 0.79$) and VLDL-cholesterol ($P < 0.05$; $r = 0.47$) concentrations. Sex had no significant effect on the concentrations of cholesterol, triglycerides, total lipid, HDL-cholesterol, LDL-cholesterol and VLDL-cholesterol.

Table 1. Mean (\pm SEM) of serum lipids and lipoproteins of adult Caspian miniature horses in different age groups (n = 50)

Age (Yrs)	N° of horses (both sexes)	Cholesterol (mmol/L)	Triglyceride (mmol/L)	Total lipid (g/L)	HDL cholesterol (mmol/L)	LDL cholesterol (mmol/L)	VLDL cholesterol (mmol/L)
1.5 - 3	23	3.09 \pm 0.31 ^a	0.14 ^a \pm 0.07	2.36 ^a \pm 0.24	1.07 ^a \pm 0.12	2.18 ^a \pm 0.30	0.028 ^a \pm 0.01
3 - 5	19	4.06 ^b \pm 0.29	0.22 ^b \pm 0.09	2.96 ^b \pm 0.27	1.23 ^b \pm 0.15	2.89 ^b \pm 0.29	0.044 ^b \pm 0.02
>5	8	5.07 ^c \pm 0.33	0.28 ^c \pm 0.08	3.27 ^c \pm 0.21	1.39 ^c \pm 0.13	3.78 ^c \pm 0.32	0.056 ^c \pm 0.01

a, b, c - values with different letters in a column indicate significant differences ($P < 0.05$); HDL - high density lipoprotein; LDL - low density lipoprotein; VLDL - very low density lipoprotein

Table 2. Mean (\pm SEM) of serum lipids and lipoproteins of adult male and female Caspian miniature horses (n = 50)

Sex	N° of horses (both sexes)	Cholesterol (mmol/L)	Triglyceride (mmol/L)	Total lipid (g/L)	HDL cholesterol (mmol/L)	LDL cholesterol (mmol/L)	VLDL cholesterol (mmol/L)
Male	32	4.07 \pm 0.33	0.21 \pm 0.08	2.86 \pm 0.22	1.23 \pm 0.13	2.95 \pm 0.29	0.042 \pm 0.01
Female	18	4.04 \pm 0.29	0.20 \pm 0.09	2.83 \pm 0.24	1.22 \pm 0.14	2.92 \pm 0.31	0.040 \pm 0.01

HDL - high density lipoprotein; LDL - low density lipoprotein; VLDL - very low density lipoprotein

Discussion

The concentration of serum cholesterol in Caspian miniature horses was higher than the values reported for Arab, Thoroughbred and Standardbred horses (AFIFI et al., 1979; KANEKO, 1989; ROBINSON, 1997), but similar to the value reported for Turkoman horses (NAZIFI et al., 2003). The concentration of triglycerides in the serum of Caspian miniature horses was lower than the values reported for horses and ponies (BAUER et al., 1990; DUNCAN et al., 1994; ROSE and HODGSON, 2000) but similar to the value reported for Turkoman horses (NAZIFI et al., 2003). There is little information concerning serum total lipid in horses. The concentration of total lipid in the serum of Caspian miniature horses was similar to the value reported for Turkoman horses (NAZIFI et al., 2003). The concentration of total lipid in the serum of Caspian miniature horses was lower than the values reported in goats (CASTRO et al., 1977; PUGLIESE et al., 1982) and in camels (NAZIFI et al., 2000), but similar to the values reported in several species by PRASAD and RAJYA (1979). Again, there is a paucity of information about serum lipoproteins in horses. The concentration of serum lipoproteins (HDL, LDL and VLDL) in Caspian miniature horses was similar to the values reported for Turkoman horses (NAZIFI et al., 2003). This study shows that the concentration of lipoproteins (HDL, LDL and VLDL) in the serum of Caspian miniature horses was lower than the values reported for dogs (BARRIE et al., 1993), but higher than the values reported for camels (NAZIFI et al., 2000). There are large species differences in lipoprotein profiles and the percentage of total cholesterol carried by each lipoprotein class. Cats and dogs have the highest percentage of HDL of the domestic species characterized to date. Cattle have approximately equal HDL and LDL cholesterol concentrations. In sheep and horses, the majority of cholesterol circulates as HDL. In pigs, more than half of the total cholesterol circulates as LDL and VLDL combined (LATIMER et al., 2003). These species differences are due to nutritional and metabolic differences. Nutritional and metabolic alterations of lipoprotein metabolism are important in equine species. An increase in resting muscle glycogen concentration has been reported by a number of workers following fat supplementation in the horse (MEYERS et al., 1989; HARKINS et al., 1992; SCOTT et al., 1992).

In this respect, ORME et al. (1997) reported that following 10 weeks of fat supplementation, thoroughbred horses exhibited a significant decrease in postprandial plasma triacylglycerol concentration. Similarly, SCHMIDT et al. (2001) reported that feeding the fat-enriched diet, independently of its energy content, led to a significant decrease in plasma triglycerides, associated with a mean 50% increase of plasma lipoprotein lipase activity.

Age had a significant effect on the serum concentration of cholesterol, triglyceride, total lipid, HDL cholesterol, LDL cholesterol and VLDL cholesterol of Caspian miniature horses, the values being higher in older animals. In contrast, WASTON (1993a) reported that plasma cholesterol, triglyceride and LDL concentrations are significantly higher in suckling foals than non-fasted adult ponies. BENNIS et al. (1992) reported that in kids the concentration of all lipids was similar to that in mature goats. In Iranian male goats, with an increase in age there were significant decreases in the triglycerides and VLDL-cholesterol (NAZIFI et al., 2002). HUGI and BLUM (1997) reported that, in calves, the concentration of cholesterol increased transiently with age, but triglycerides did not show a consistent change. NAZIFI et al. (2000) reported that in dromedary camels the concentrations of cholesterol, triglyceride, total lipid, HDL cholesterol and VLDL cholesterol increased and that the concentration of LDL cholesterol decreased with increasing age. In humans, BRAUNWALD (1995) and KLEINVELD (1996) reported that there was a statistically significant increase in the concentrations of serum cholesterol and triglyceride in advanced age. NOGUCHI (1993) reported that the concentrations of LDL and VLDL increased and that the concentration of HDL decreased with increasing age.

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SAŽETAK

Uzorci krvi uzeti su iz jugularne vene 50 klinički zdravih kaspjskih minijaturnih konja i razvrstani s obzirom na njihovu dob (1,5-3, 3-5 i >5 godina) i spol. Istraživane su razlike u serumskoj koncentraciji kolesterola,

triglicerida, ukupnih lipida, lipoproteina vrlo niske gustoće (VLDL-kolesterol), lipoproteina niske gustoće (LDL-kolesterol) i lipoproteina visoke gustoće (HDL-kolesterol). Koncentracije kolesterola, triglicerida, ukupnih lipida, HDL-kolesterola, LDL-kolesterola i VLDL-kolesterola u različitim dobnih skupina bile su signifikantno različite ($P < 0,05$). Sa starošću životinja značajno ($P < 0,05$) se povećavala i koncentracija kolesterola, triglicerida, ukupnih lipida, HDL-kolesterola, LDL-kolesterola i VLDL-kolesterola. Spol nije utjecao na značajnije razlike u koncentraciji navedenih parametara.

Ključne riječi: lipidi, lipoproteini, serum, kaspijski minijaturni konj
