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Investigation of the relationship of asprosin and cardiac troponin response with some physiological and biochemical parameters in Anatolian native horse

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Summary: Asprosin is a recently discovered glucogenic hormone released from starvation-induced white adipose tissue and amongst others it protects against hyperinsulinism associated with metabolic syndrome. The aim of this study was to examine the effect of age, gender and season on metabolic (asprosin) and cardiovascular (cardiac troponin, I (cTnI), CK, CK-MB) function markers and their relationship with some biochemical parameters in Anatolian native horse breed. This study was carried out between February–August 2020. A total of 40 horses was used for this purpose, including 20 mares and 20 stallions in the age range of 1–6, 7–12. Serum asprosin level, for the winter season in stallions and mares aged 1–6 and 7–12 years respectively 8.9, 8.36; 14.08, 13.58 ng/ml were as determined. In addition, for the summer season respectively 17.98, 18.68; 22.22, 21.32 ng/ml were detected and no difference between the groups (P>0.05). On the other hand, serum cTnI levels for the winter season in stallions and mares aged 1–6 and 7–12 years respectively 0.014, 0.018; 0.016, 0.02 ng/ml were as determined. In addition, for the summer season respectively 0.012, 0.016; 0.014, 0.018 ng/ml were detected. It was shown that the serum biochemical parameters and levels of cTnI levels in the domestic horse breed the horses belonging to the Anatolian varied depending on age, gender, and season ($p < 0.05$) but the effect of hormone levels serum asprosin wasn't found statistically significant ($p>0.05$).Our results showed that cTnI could be a useful parameter in the examination of the cardiovascular system status of horses.

Keywords: asprosin, horse, Anatolian gender, cardiac troponin I, season, age

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Introduction

Asprosin is a recently discovered glucogenic hormone released from starvation-induced white adipose tissue, located at chromosome locus 15q21.1, 235 kb long, encoded by two exons (exon 65 and exon 66) of the fibrilin 1 (FBN1) gene (*Romere* et al. 2016, *Yuan* et al. 2020). Asprosin activates the G proteins cyclic adenosine monophosphate (cAMP) dependent protein kinase (PKA) pathway when the glucose level decreases, enabling rapid glucose release from the liver into the circulation. It also protects against hyperinsulinism associated with metabolic syndrome (*Romere* et al. 2016).

Troponins are regulatory proteins that have a key role in muscle contraction, with three sub-forms troponin C (TnC), troponin I (TnI) and troponin T (TnT). Cardiac troponin (cTnI) is a 22.5 kDa molecular weight cardiac muscle protein involved in myocardial contraction by controlling the interaction between actin and myosin through calcium (*Freda* et al. 2002, *Parmacek* and *Solaro* 2004). cTnI is released from cardiac myocytes into plasma in response after cardiac injury. Therefore, it is a high cardiospecific cardiac marker used in the evaluation of myocardial damage and diseases (*Adams* et al. 1994, *Fennell* and *Forbes* 2009, *Wu* et al. 1996). In addition to confirming its use in horses, there are studies reporting that cTnI is increased in heart diseases (*Fennell* and *Forbes* 2009, *Schwarzwald* et al. 2003). It is reported that asprosin protects mesenchymal stromal cells by activating the ERK1/2-SOD2 pathway, inhibiting apoptosis caused by oxidative stress (*Zhang* et al. 2019). CK-MB, AST, ALT, Lactate Dehydrogenase (LDH) are some parameters used as a prognostic marker in cardiovascular diseases and myocardial damage (*Danese* and *Montagnana* 2016, *Mythili* and *Malathi* 2015).

The Anatolian native horse is among the domestic horse breeds specific to Turkey. It is a horse breed that is resistant to harsh climatic conditions and is used for riding and transportation as well as agricultural activities (*Koçkar* 2012, *Taşkın* and *Koçak* 2010).

Muscle damage and the level of damage vary depending on factors such as age, gender, race, and especially the training program (*Chiaradia* et. 1998). On the other hand, although the impact of age and seasonal changes on cardiovascular disorders is well understood so far (*Mikić* et al. 2018). No study investigating the effect of age, gender and season of asprosin level, which is reported to have therapeutic potential in cTnI and metabolic syndrome, has been found.

In this study, it was aimed to determine the asprosin and cardiac troponin levels in the serum of the Anatolian native horse, as well as to examine the relationship with some biochemical parameters depending on age, gender and seasonal changes.

Material and method

The study was carried out in 11 horse farms in the center and districts of Bayburt, raised under intensive conditions and regularly followed up. In the research, 40 horses belonging to the Anatolian horse breed, between the ages of 1–6 and 7–12, were created as animal material. The mean body condition score of horses in the 1–6 age group [BCS] is 5.0; mean BCS is 4.7 in horses in the 7–12 age group. Cardiovascular system examination of horses was evaluated by auscultation technique. Horses that were found to have a regular heart rhythm by resting with a stethoscope and had a clinically healthy appearance as a result of routine clinical examination were included in the study. The animals used in the study were included in the study group on the one-hoofed animal identification documents by using a random trial order so that the total live weight average of the groups was equal. Horses were divided into 4 groups in the age and gender category. The first group was mare horses between the ages of 1–6 ($n = 5$), the second group was stallion horses between the ages of 1–6 ($n = 5$); the third group is mare horses between the ages of 7–12 ($n = 5$); the fourth group was composed of stallion horses ($n = 5$) between the ages of 7–12. Blood was taken from the same animals in these groups once in winter and summer. Serums obtained from blood samples were stored at –80 °C. Serum asprosine and cTnI levels were determined by ELISA method. During the research, attention was paid to keep the environment and feed factor (Table1) the same. Analysis of the content of feeds in this study was analyzed according to standard *AOAC* methods (*AOAC*, 2005).

Collection of serum samples

10 ml of blood samples were taken from the cervical vein (vena jugularis) of the horses comprising the study groups into anti-coagulant-free tubes (Vacuette® Tube 9 ml Z Serum Clot Activator). The blood taken into the tubes was centrifuged in the laboratory in a cooled centrifuge (NF 1200R, NÜVE, Ankara, Turkey) at 3000 rpm for 10 minutes. The serums obtained were transferred to sterile Eppendorf tubes. It was stored in deep freezers set at –80 °C until laboratory analyzes were carried out.

Table 1 Basic ration nutrient content for horses (g/kg) | *Basisration Nährstoffinhalte für Pferde (g/kg)*

Ration content	Ration quantity (kg) Mare
Wheat Straw	–
Meadow Grass	–
Barley Crushed	2
Cavdar Crush	1.5
Clover	6
Oat	4

Biochemical analysis

In the study, ALT, AST, ALP, LDH, and VLDL, LDL, HDL, TG, total cholesterol, CK, CK-MB and glucose, GGT levels were measured with a fully automatic biochemical device Cobas 8000 (Roche, Germany).

Measurement of serum asprosin level

The minimum detectable concentration of the kit used to measure the asprosin level in the blood serum samples of horses obtained as a result of the study was reported as 13 pg/ml. The ELISA kit is specified in the manufacturer's catalog using the species-specific Horse Cardiac Troponin I (cTnI) ELISA Kit, Product code: SG-53127, China), determination 31.3 pg/ml–4000 pg/ml, intra assay coefficient 8.0 %, inter assay coefficient 10.0 %. It was studied in accordance with the procedure.

Measurement of serum cTnI level

As a result of the study, the minimum detectable concentration of kit used to measure asprosin levels in blood serum samples of horses was reported as 2.1 ng/mL. ELISA kit, species-specific Asprosin Horse (Horse Asprosin ELISA Kit product code: SG-53129, China), determination 7.80 ng/mL–500ng/mL, intra-assay coefficient % 8.0 inter assay coefficient % in accordance with the procedure specified in the manufacturer's catalog was studied using 10.0.

Statistical analysis

The data obtained as a result of the study were statistically analyzed by Duncan test (glucose, CK-MB, ALP, ALT and LDH) at general Linear Model univariate in IBM SPSS v25 statistical program. The analysis was performed in a fully factorial environment with 3 predictor variables ($2 \times 2 \times 2$) according to a fully randomized design. The variables studied and their interactions with the statistical model are given below:

$$Yijkl = \mu + \alpha i + \beta j + \gamma k + (\alpha\beta)ij + (\alpha\gamma)ik + (\beta\gamma)jk + (\alpha\beta\gamma)ijk + \varepsilon\ ijkl.$$

Here, Y is μ: average, α gender, age, and γ season effect. Measurements that did not have normal distribution (cTnI, Aspirosin, VLDL, LDL, HDL, TG, AST, GGT, CK and Cholesterol) were analyzed with the Mann Whitney U nonparametric test. All significant differences were evaluated with tests at the $p < 0.05$ level.

Results

The results of evaluating the effect of age, gender and season on the parameters that make up the biochemical analysis of the Anatolian horse breed are shown in Tables 2 and 3. The mean serum asprosin hormone levels of mare and stallion

Table 2 Serum asprosin, cTnI and some biochemical parameters and statistical comparisons (mean ± SEM) | *Serum-Asprosin, cTnI und ausgewählte biochemische Parameter sowie statistische Vergleiche (Mittelwert ± SEM)*

Some biochemical values of the Anatolian native mare

Age	Asprosin (ng/ml)		cTnI (ng/ml)		VLDL (mg/dl)		LDL (mg/dl)		HDL (mg/dl)	
	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer
1–6	0.014 ± 0.01	0.012 ± 0.01	8.9 ± 0.24	8.36 ± 0.83	4.004 ± 0.11	3.774 ± 0.17	25.274 ± 1.67	24.816 ± 0.92	41.22 ± 1.59	41.06 ± 1.02
7–20	0.018 ± 0.01	0.016 ± 0.01	14.08 ± 0.51	12.48 ± 0.99	6.254 ± 0.13	6.122 ± 0.27	25.178 ± 0.38	26.228 ± 0.45	43.96 ± 0.82	44.96 ± 0.76

Some biochemical values of the Anatolian native stallion

Age	Asprosin (ng/ml)		cTnI (ng/ml)		VLDL (mg/dl)		LDL (mg/dl)		HDL (mg/dl)	
	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer
1–6	0.016 ± 0.01	0.014 ± 0.01	8.36 ± 0.50	7.78 ± 0.81	3.946 ± 0.17	3.746 ± 0.21	24.834 ± 0.92	24.634 ± 0.89	40.96 ± 1.05	40.7 ± 1.14
7–20	0.02 ± 0.01	0.018 ± 0.01	13.58 ± 0.58	11.7 ± 1.20	6.116 ± 0.48	6.068 ± 0.54	25.19 ± 0.38	26.246 ± 0.44	44.08 ± 0.84	45.04 ± 0.72

Variation (p values)

	Asprosin	cTnI	VLDL	LDL	HDL
Age*Season	0.52	0.00	0.00	0.00	0.00
Gender*Season	1.00	0.10	0.50	0.5	0.55
Gender *Age	0.05	0.00	0.00	0.07	0.00
Season	0.32	0.33	0.84	0.89	1.00
Age	0.06	0.00	0.00	0.00	0.00
Gender	0.32	0.06	0.30	0.13	0.40
Age*Season*Gender	0.41	0.00	0.00	0.17	0.00

Main effect

	Asprosin	cTnI	VLDL	LDL	HDL
Stallion	0.015 ± 0.01	10.08 ± 2.27	4.9275 ± 1.23	25.481 ± 1.02	42.94 ± 2.28
Mare	0.015 ± 0.01	11.23 ± 2.71	5.08 ± 1.16	25.119 ± 0.92	42.555 ± 1.2

Table 2 Part 2 Serum asprosin, cTnI and some biochemical parameters and statistical comparisons (mean±SEM) | *Serum-Asprosin, cTnI und ausgewählte biochemische Parameter sowie statistische Vergleiche (Mittelwert ± SEM)*

Age	TG (mg/dl)		Total cholesterol (mg/dl)		AST (IU/L)		GGT (IU/L)		CK (IU/L)	
Some biochemical values of the Anatolian native mare										
	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer
1–6	20.88 ± 0.92	19.86 ± 0.45	92.32 ± 1.13	89.44 ± 0.58	307.24 ± 4.00	309.06 ± 5.41	20.1 ± 1.01	20.22 ± 0.83	193.74 ± 1.75	200.46 ± 1.42
7–20	24.7 ± 0.82	24.1 ± 0.87	87.72 ± 1.54	86.68 ± 0.91	306.22 ± 2.39	306.92 ± 2.43	21.16 ± 1.31	22.08 ± 1.59	205.1 ± 3.96	207.86 ± 2.15
Some biochemical values of the Anatolian native stallion										
Age	TG (mg/dl)		Total cholesterol (mg/dl)		AST (IU/L)		GGT (IU/L)		CK (IU/L)	
	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer
1–6	21.1 ± 0.9	20.02 ± 0.53	88.04 ± 1.08	87.16 ± 0.87	308.72 ± 9.32	311.72 ± 3.94	19.92 ± 0.44	19.98 ± 0.78	200.72 ± 3.38	205.78 ± 3.37
7–20	24.84 ± 0.77	24.34 ± 0.86	86.32 ± 0.71	86.24 ± 0.48	308.86 ± 0.82	308.18 ± 1.39	20.76 ± 1.27	21.74 ± 1.51	208.96 ± 0.87	210.38 ± 0.88
Variation (p values)										
Age*Season	0.00		0.00		0.65		0.00		0.00	
Gender*Season	0.50		0.01		0.04		0.68		0.01	
Gender *Age	0.00		0.00		0.82		0.05		0.00	
Season	0.58		0.01		0.16		0.42		0.01	
Age	0.00		0.00		0.39		0.00		0.00	
Gender	0.17		0.10		0.21		0.22		0.03	
Age*Season*Gender	0.00		0.00		0.75		0.04		0.00	
Main effect										
Stallion	22.08 ± 2.29		87.38 ± 1.43		308.97 ± 3.79		21.005 ± 1.47		206.12 ± 4.24	
Mare	22.88 ± 2.09		88.6 ± 2.53		307.76 ± 4.92		20.485 ± 1.10		202.13 ± 6.33	

Table 3 Some biochemical parameters and statistical comparisons (mean ± SH) | *Einige biochemische Parameter und statistische Vergleiche (Mittelwert ± SH)*

	Glucose (mg/dl)				CKMB (U/L)				ALP (IU/L)				ALT (IU/L)				LDH (IU/L)			
	Winter		Summer		Winter		Summer		Winter		Summer		Winter		Summer		Winter		Summer	
	Mare	Stallion	Mare	Stallion	Mare	Stallion	Mare	Stallion	Mare	Stallion	Mare	Stallion	Mare	Stallion	Mare	Stallion	Mare	Stallion	Mare	Stallion
1–7	86.08	87.56	85.65	86.43	164.22	170.92	164.28	177.30	253.66	254.02	246.10	247.96	8.54	8.52	9.32	9.18	243.32	242.20	242.96	241.46
7–12	85.85	87.12	85.45	86.36	171.96	179.32	171.98	179.38	252.86	253.28	248.64	248.96	8.88	8.68	9.24	9.14	244.52	244.56	244.02	243.62
SEM	0.39				2.07				2.33				0.15				1.29			
Effects																				
Effect of Age (1–7)	86.429				169.18				250.43				8.89				242.485			
Effect of Age (7–12)	86.429				175.66				250.93				8.985				244.18			
Effect of Season (Winter)	86.651				171.605				253.45				8.665				243.65			
Effect of Season (Summer)	85.974				173.235				247.91				9.22				243.015			
Effect of gender (Mare)	85.759				168.11				250.31				8.995				243.71			
Effect of gender (Stallion)	86.866				176.73				251.05				8.88				242.96			
SEM	0.196				1.036				1.167				0.073				0.648			
Interaction Effect (P values)																				
Gender	0.00				0.00				0.65				0.27				0.42			
Age	0.41				0.00				0.76				0.36				0.07			
Season	0.02				0.27				0.00				0,00				0.49			
Gender * Age	0.95				0.40				0.82				0.73				0.54			
Gender * Season	0.35				0.29				0.83				0.96				0.82			
Age * Season	0.72				0.29				0.44				0.14				0.92			
Gender * Age* Season	0.76				0.29				0.81				0.59				0.98			

horses between 1–6 and 7–12 years of age were 8.9, 8.36; 14.08, 13.58 ng/ml for winter, respectively, while in summer these values were 8.36, 7.78; 12.48, 11.7 ng/ml, respectively (Table 2). On the other hand, mean cTnI levels of mare and stallion horses in the 1–6; 7–12 age range were 0.014, 0.018; 0.016, 0.02 ng/ml for winter, while in summer these values were 0.012, 0.016; 0.014, 0.018 ng/ml, respectively (Table 2). As a result of the study, no statistically significant difference was found in mean serum asprosin hormone levels, age, gender, and season ($p>0.05$). On the other hand, it was determined that there was a statistically significant effect on the mean serum cTnI level by the interactions of age with the season, gender, and age, only age (season*gender*age) ($p<0.05$).It was determined that age and season factors were statistically significant on VLDL, LDL, HDL, TG, total cholesterol, GGT, glucose, CK, CK-MB levels ($P<0.05$).

Discussion

Metabolic syndrome, insulin resistance, impaired glucose balance, dyslipidemia is a metabolic disorder characterized by biochemical and clinical manifestations in which some or all of the parameters of obesity, hypertension are present together. The metabolic syndrome of horses is a metabolic disease of equines characterized by insulin resistance, extreme obesity, endocrine pathological condition associated with regional steatosis, and laminitis (*Frank* and *Tadros* 2014). In metabolic syndrome, adipokine levels vary in circulatory blood as well as systemic inflammation (*Frank* et al. 2010, *Vick* et al. 2007). Asprosin is one of the adipokines recently discovered and reported to have therapeutic potential in metabolic syndrome (*Hoffmann* et al. 2020, *Yuan* et al. 2020). In addition, although cardiovascular disease and mortality risks of metabolic syndrome have been reported, the underlying mechanism is not fully understood (*Ju* et al. 2012). There is a close relationship between cardiac troponin I, a cardiac marker with high cardiospecificity, and metabolic syndrome in the evaluation of myocardial diseases (*Sugiura* et al. 2019).

When the results of asprosin (*Bayraktar* 2020) and cTnI in the mean serum of our study are evaluated in general, the results of the previous study are consistent (*Díaz* et al. 2014, *Nath* et al. 2012, *Schwarzwald* et al. 2003, *van der Vekens* et al. 2015). Although the studies examining the asprosin hormone values in horses are limited, we think that the different results to be obtained may justify the literature information, that the development of adipose tissue varies according to race, age and gender (*Ravaglia* et al. 1999) and creatine kinase-myocardial isoenzyme (CK-MB) were not significantly influenced by age, season, gender, and seasonal interactions ($p>0.05$). The current results are consistent with the literature data (*Altıntaş* and *Fidancı* 1993, *Kaneko* et al. 1997).On the other hand, when examined in terms of creatine kinase (CK), the effect of age, season, gender, season, gender, age and age, gender and seasonal interactions on serum CK levels. It was found statistically significant ($p<0.05$).

In the presented study, serum VLDL, LDL, HDL (*Nazifi* et al. 2003, *Nazifi* et al. 2005), GGT, glucose (*Altıntaş* and *Fidancı* 1993, *Kaneko* et al. 1997), AST, ALT, LDH, ALP (*Kaneko* 1989, *Rubino* et al. 1989, *Oktay* and *Eren* 2014), TG (*Mohri* et al. 2005, *Oktay* and *Eren* 2014), total cholesterol leves (*Altıntaş* and *Fidancı* 1993, *Kaneko* et al. 1997) are within normal reference ranges. It showed compatibility with reported study results. However, TG and total cholesterol levels were determined by *Özcan* et al. (2002). The difference in the results reported by *Özcan* et al. (2002) is thought to be shaped by the variability in lipid metabolism due to race.

Conclusion

As a result of this research, the effects of age, gender and season on serum asprosin and cTnI levels and some biochemical parameters in horses belonging to Anatolian native horse breed were revealed. We think that there may be new and useful biomarker that can be measured in serum for the evaluation and follow-up of many metabolic and cardiovascular diseases and disorders such as metabolic syndrome in horses.

Animal welfare statement

Before starting the current study, ethics committee approval was obtained from the Bayburt University Local Ethics Committee for the study (Decision date and number: 27.03.2019/01). The study was carried out in accordance with ethical principles and rules, protecting animal welfare and rights.

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