

## **Insulin Resistance and its Relevance with Age, Sex, Season and Obesity in Darehshori Horse Breed**

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### **Abstract**

**BACKGROUND:** Equine metabolic syndrome is an endocrinopathy that affects different breeds of horses and ponies. This metabolic disorder is a multifactorial syndrome and it is primarily linked to obesity, dyslipidemia and insulin resistance.

**OBJECTIVES:** The present study was performed to evaluate insulin resistance in Darehshori horses with regards to age, sex, season and obesity.

**METHODS:** Twelve adult stallions and 14 non-pregnant Darehshori breed mares were selected between January and November 2015. Fourteen horses were under and others were above 10 years old. Body condition scores of the animals were estimated based on Hennekes' 1 to 9 scoring system, thus, 1 is poor and 9 is extremely fat horse. Body condition score of 12 horses was under 5 and the others above 5. The horses were studied during a period of one year, every 45 days and blood sampling and body condition scoring were performed at each time. Insulin resistance was assessed based on screening test. Serum concentrations of glucose, insulin and leptin were determined.

**RESULTS:** This study comprehensively used multiple detailed parameters to scan thoroughly the concept of insulin resistance in Darehshori horses on a long term basis. The levels of insulin, glucose and leptin in aged and obese stallions in warmer months were significantly higher than the others and reference range ( $P < 0.05$ ), hence, insulin resistance was detected in these groups of studied animals.

**CONCLUSIONS:** These findings represented insulin resistance phenomenon in a specific breed in Iran. The physiologic characteristics of this breed may be different from other ones and the conditions in Iran such as climate are different from other countries. Hence, it should be considered that Darehshori horses have unique characteristics compared with other breeds. The study may be used as a guideline for evaluating the insulin resistance in Darehshori horses.

**KEYWORDS:** Darehshori horse, glucose, insulin, insulin resistance, leptin

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## Introduction

The concept of equine metabolic syndrome was first suggested by Johnson (Johnson, 2002). In human medicine, metabolic syndrome refers to a set of risk factors that predict the risk of cardiovascular disease, including obesity, glucose intolerance, insulin resistance, dyslipidemia, microalbuminuria and hypertension (Landsberg et al., 2013). Increased adiposity, hyperinsulinemia, and insulin resistance are the three principal components of equine metabolic syndrome. It is difficult to separate those factors from one another. Hyperinsulinemia is detected in most insulin resistant horses and affected animals are usually obese or exhibit regional adiposity (Frank and Tadros, 2014). Normal actions of insulin include inhibition of gluconeogenesis and lipolysis and stimulation of glycogen synthesis. Insulin resistance is defined as a reduction in the action of insulin on target tissues. Mechanisms of insulin resistance include defects in the insulin receptor, insulin signaling pathways or glucose transporter 4 (GLUT4) synthesis, translocation or function (Samuel et al., 2016). There are different suggested methods to identify the insulin resistant horses. Screening tests are the practical methods and dynamic diagnostic tests containing combined glucose-insulin test and oral sugar test are also other suggested methods (Frank and Tadros, 2014).

Equine metabolic syndrome and insulin resistance occur most commonly in pony, Morgan, Paso Fino, Arabian, Saddlebred, Quarter and Tennessee Walking breeds. Most horses and ponies with equine metabolic syndrome are obese and owners often describe them as easy keepers. Environmental issues such as overfeeding and lack of exercise contribute to obesity and these problems are increasing with modern management practices (Frank

and Tadros, 2014).

To the best of the author's knowledge, there is no information about equine metabolic syndrome and insulin resistance phenomenon in Darehshori horses as an originated breed from Iran. Hence, the present study was carried out by the screening method to evaluate the presence of insulin resistance and its probable relation with age, sex, season and obesity in this breed, during one year.

## Materials and Methods

This project was carried out from January till November 2015 on 26 Darehshori horses (12 adult stallions and 14 non-pregnant, non-lactating adult mares) at several equestrian clubs around Shiraz, southwest Iran. Fourteen horses were under and others were above 10 years old. All of them were in resting period, kept in individual stalls and fed with balanced rations including alfalfa hay, corn silage and barley grain. The horses were clinically healthy, had no history of debilitating diseases, laminitis and were free from internal and external parasites based on laboratory parasitic examinations and routine anti parasitic programs. Body condition scores of the animals were estimated based on Henneke et al. (1983), 1 to 9 scoring system. The Hennekes' horse body condition scoring system is a numerical scale used to evaluate the amount of fat on a horse's body. It was first published by Henneke et al. at 1983 to create a universal scale to assess horses' bodyweight. It is a standardized system that can be used across all breeds without specialized equipment; condition is assessed visually and by palpation. Scores range from 1 to 9 which refer to poor, very thin, thin, moderately thin, moderate, moderately fleshy, fleshy, fat and extremely fat, hence, one being poor

and nine being extremely fat. The ideal range for most horses is from 4 to 6. The system is based on both visual appraisal and palpable fat which cover the six major points of the horse including neck, shoulder, ribs, withers, lumbar vertebrae and tail head. Body condition score of 12 horses was under 5 and the others were above 5. The horses were studied during one year, every 45 days and blood sampling and body condition scoring were performed at each time. The first sampling was carried out at 1 January 2015 and was continued to 15 November 2015.

Insulin resistance phenomenon was assessed based on screening test (Frank and Tadros, 2014). According to this method, we left only one flake of hay after 10:00 PM for each horse and blood was collected at 06:00 AM the next morning. Evaluating the serum fasting glucose, insulin and leptin levels could reflect the insulin resistance or sensitivity. According to this procedure, if fasting glucose, insulin and leptin were respectively higher than 110 mg/dL, 20  $\mu$ U/mL and 7 ng/mL, it could be concluded that the horse was insulin resistant (Frank and Tadros, 2014).

Blood samples were collected via jugular venipuncture from all animals, every 45 days, in plain tubes. Immediately after blood collection, sera were separated by centrifugation for 10 minutes at 3000 rpm and stored at -22 °C until assayed. Glucose was assayed by an enzymatic (glucose oxidase) colorimetric method (ZistChem®, Tehran, Iran). Insulin and leptin were measured by equine ELISA kits (Eastbiopharm®, China). Reference number of equine insulin and leptin ELISA kits were E20160801045 and E20160801046. These analyses were performed by Stat Fax® 2100.

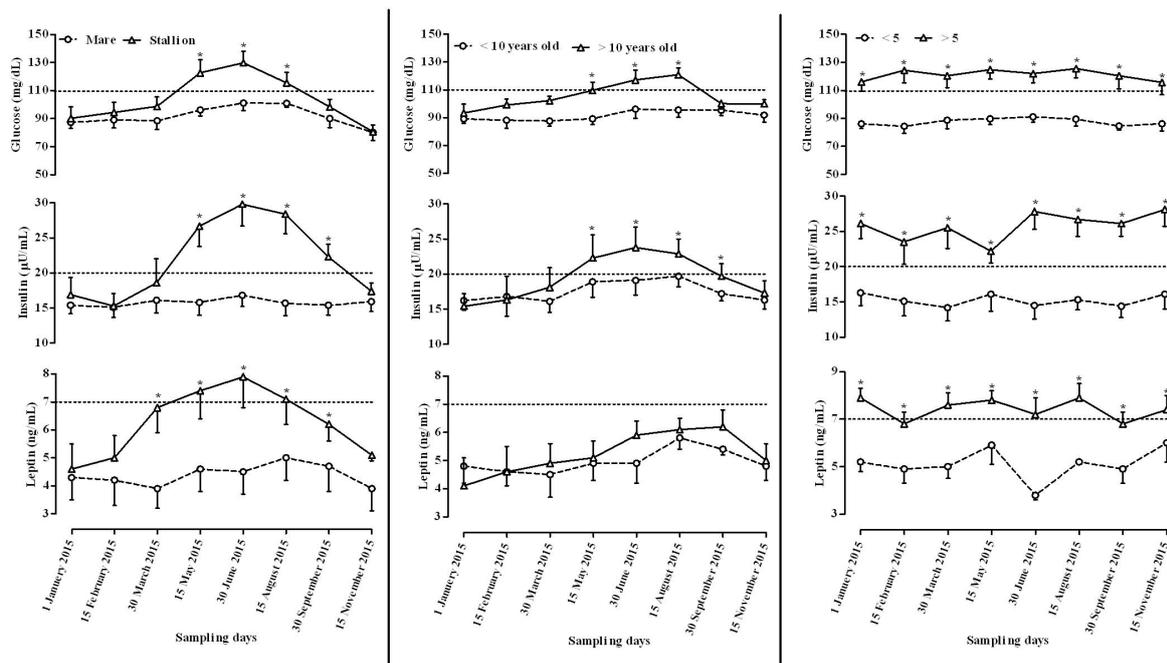
All data are presented as mean  $\pm$  standard deviation (SD). Differences between the av-

erage concentrations of each serological factor between groups (age, sex and body condition scores) on similar days were analyzed by repeated measures ANOVA. Repeated measures ANOVA was also used to evaluate the changing patterns of each factor during the year. The statistical analyses were performed by using SPSS 20 (SPSS Inc, Chicago, Illinois). The level of significance was set at  $P$ -value $<0.05$ .

## Results

The results of the screening test, every 45 days during a year for insulin resistance based on age, sex and body condition score are presented in Fig. 1. Based on Frank and Tadros (2014), fasting glucose, insulin and leptin at 110 mg/dL, 20  $\mu$ U/mL and 7 ng/mL, respectively, are the cut-off points and higher values indicate insulin resistant horses. Horizontal dotted lines at these limits were used to better identify the insulin resistance phenomenon at each sampling day.

Glucose levels in aged horses (above 10 years old) were significantly higher than younger ones mainly in warmer months (May till August;  $P<0.05$ ; Fig. 1). Glucose values in older horses in warmer months were higher than the cut-off point for insulin resistance. The glucose concentrations in younger horses were under the cut-off point during the period of the study. In the stallions, glucose was significantly higher than the mares in warmer months ( $P<0.05$ ) and its levels were above the cut-off point of insulin resistance. Glucose in mares was under 110 mg/dL during the study (Fig. 1). The fasting blood glucose in the horses with greater body condition scores was significantly higher ( $P<0.05$ ). The glucose levels following screening test in the horses with higher body condition scores were above the cut-off



**Figure 1.** The results of evaluating the serum glucose, insulin and leptin concentrations according to the insulin resistance screening test in Darehshori horses, based on age, sex and body condition score at every 45 day samplings, during one year. Fasting blood glucose, insulin and leptin at 110 mg/dL, 20 μU/mL and 7 ng/mL, are the cut-off points of insulin resistance, respectively. Higher values indicate insulin resistant horses. Stars indicate significant differences ( $P < 0.05$ ).

point of insulin resistance at all studied days (Fig. 1). Based on the fasting blood glucose assay for the screening testing of insulin resistance, it may be suggested that the stallions and older horses were insulin resistant in warmer months. However, obese Darehshori horses had insulin resistance during the study period (Fig. 1).

Insulin levels in older horses were significantly higher than younger ones in warmer months ( $P < 0.05$ ; Fig. 1). Its levels in older horses in warmer months were higher than cut-off point of insulin resistance. The insulin values in younger horses were under cut-off point during the study. The results of the repeated measures ANOVA showed that insulin was increased significantly following the climate becoming warmer in both age groups ( $P < 0.05$ , Fig. 1). In stallions, insulin was significantly higher than mares in warmer months ( $P < 0.05$ ) and its levels were

above the cut-off point of insulin resistance. Insulin in mares was under 20 μU/mL during the study (Fig. 1). Insulin levels in the horses with greater body condition scores were significantly higher than other ones ( $P < 0.05$ ). The insulin levels following screening test in the horses with high body condition scores were above the cut-off point of insulin resistance during the study (Fig. 1). Based on evaluating the insulin for the screening test of insulin resistance, it may be proposed that the stallions and older Darehshori horses were insulin resistant in warmer months. However, obese horses had insulin resistance during the year.

The results of the repeated measures ANOVA showed that leptin increased significantly following the the climate becoming warmer in both age groups ( $P < 0.05$ , Fig. 1). There were no statistical differences between old and young horses ( $P > 0.05$ ). Leptin in both

age groups in warmer months was higher, but its levels were under cut-off point of insulin resistance during the study (Fig. 1). In stallions, leptin was significantly higher than mares in warmer months ( $P<0.05$ ) and its levels were above the cut-off point of insulin resistance (Fig. 1). Leptin in mares was under 7 ng/mL on all of the studied days (Fig. 1). Leptin levels in the horses with greater body condition scores were significantly higher than other ones ( $P<0.05$ ). The leptin values following screening testing in horses with high body condition scores were above the cut-off point of insulin resistance at all times (Fig. 1). Based on the leptin assay for the screening test of insulin resistance, it may be suggested that the stallions and older horses were insulin resistant in warmer months and obese Darehshori horses had insulin resistance during the whole year.

## Discussion

Insulin is synthesized by the pancreatic  $\beta$  cells and is primarily a glucose storage hormone because it facilitates cellular glucose uptake, promotes glycogenesis, and inhibits gluconeogenesis (Sharabi et al., 2015). Insulin secretion increases in response to increased blood glucose concentrations to maintain normoglycemia by stimulating cellular glucose uptake, glycogenesis, and fatty acid synthesis, as well as reducing glucose production by decreasing gluconeogenesis, lipolysis and proteolysis (Samuel and Shulman, 2016).

Insulin resistance is defined as a reduction in the action of insulin on target tissues (Frank and Tadros, 2014). Mechanisms of insulin resistance include defects in the insulin receptor, insulin signaling pathways, or glucose transporter 4 (GLUT4) synthesis, translocation, or function. One important action

of insulin is to stimulate glucose transport into cells, and this occurs rapidly as GLUT4 proteins translocate to cell membranes. Results of a recent study indicate that GLUT4 translocation is impaired in insulin-resistant horses (Samuel and Shulman, 2016). Insulin resistance screening testing is a simple and practical method to diagnose the equine metabolic syndrome (Frank and Tadros, 2014). In this method, fasting blood glucose and insulin concentrations should be measured to screen for hyperglycemia and hyperinsulinemia, which serve as indicators of insulin resistance. Leptin measurements are also suitable to evaluate the fat metabolism. During the screening tests, blood glucose concentrations are within reference range in most insulin-resistant horses because euglycemia is maintained through increased pancreatic insulin secretion. However, glucose concentrations should always be measured to detect uncompensated insulin resistance or diabetes mellitus. Some of these patients can only be identified by detecting hyperglycemia because insulin concentrations have returned to reference range as a result of pancreatic insufficiency (Frank and Tadros, 2014).

At present, the most useful screening test for insulin resistance is the resting insulin concentration, which must be performed after a short fast to minimize the impact of feeding (Anhê et al., 2015). As with many tests, the result is more likely to be a true positive the further it falls outside of reference range. A markedly elevated fasting insulin concentration therefore serves as a good indication of insulin resistance. However, it is more difficult to interpret results that are closer to reference range and breed-specific ranges are needed to improve accuracy (Frank and Tadros, 2014). The present study tried to find out the insulin resistant Darehshori horses at dif-

ferent situations such as age, sex, season and obesity. Higher resting serum insulin concentrations were detected in aged stallions with obesity, suggesting that this value may be a useful screening parameter for insulin resistance. Hyperinsulinemia is a feature of insulin resistance in humans and occurs when insulin secretion from the pancreas increases to compensate for reduced response to insulin (Cerf, 2013).

Based on the results of the current research, insulin resistance was not found in younger horses in any of the seasons but older ones showed this phenomenon in warmer months (Fig. 1). Darehshori stallions were insulin resistant in warmer months only, but this metabolic disorder was not found in Darehshori mares in any of the studied seasons (Fig. 1). Results of the Frank and Tadros (2014) study indicated that resting serum insulin and leptin concentrations are useful screening parameters for insulin resistance in horses, but other factors including time of day and season must be considered when interpreting these values. Gordon and McKeever (2005) determined that glucose and insulin responses to feeding are higher in the morning. Fitzgerald and McManus (2000) determined that serum leptin concentrations were highest in mature mares during the late summer and early fall and lowest during the winter months in Kentucky. They stated that the serum insulin concentrations in obese mares across a 12-month period were also higher during the summer and early fall.

In the present study, the horses with lower body condition scores had no insulin resistance in any of the studied seasons but obese horses showed this phenomenon at all sampling months (Fig. 1). Frank and Tadros (2014) assembled a population of obese horses with insulin resistance, and these horses

had significantly greater resting glucose, insulin, and leptin concentrations than healthy non-obese horses.

Some horses are genetically predisposed to obesity because of adaptations to survival on poorer quality forages (Giles et al., 2014). According to this theory, consumption of concentrated feeds or grazing on rich pastures might therefore promote obesity in susceptible horses. Genetic and environmental factors are likely to be important in the development of obesity in horses, and it is interesting that obese insulin resistant horses are aged, which suggests that time is required for environmental factors to alter glucose metabolism (Frank and Tadros, 2014). Obesity was defined by body condition score in our study with the scale developed by Henneke et al. (1983). Obese horses in this study were insulin resistant and they had regional adiposity at those locations. An association between obesity and insulin resistance has been reported in horses (Hoffman et al., 2003). Hoffman et al. (2003) studied obese Thoroughbred geldings by use of the frequently-sampled intravenous glucose tolerance test and they concluded that mean insulin sensitivity was < 20% of the value in non-obese geldings. Obesity and lack of exercise are primary risk factors for insulin resistance in humans, and the risk of developing type 2 diabetes mellitus increases with the severity of obesity (Landsberg et al., 2013).

Adipokines are released from adipocytes and two adipokines have been examined to date in horses including leptin and adiponectin (Frank, 2018). Leptin is an adipocyte-derived hormone product of the obesity gene that influences food intake and energy use, and can be used as an indicator of energy balance. High levels of leptin increase energy expenditure and decrease food intake and

vice versa (Pan et al., 2014). Leptin stimulates the sympathetic system in brown adipose tissue and stimulates triglyceride and fatty acid cycling by increasing lipolysis and fatty acid oxidation. Leptin is secreted in proportion to fat mass, although massively obese humans seem to be resistant to leptin (Pan et al., 2014). In horses, plasma leptin is positively correlated with fat mass percentage and body condition score (Selim et al., 2015). The present study is in agreement with the results of Fitzgerald and McManus (2000) which showed that leptin has seasonal variations in young and old mares, with plasma leptin levels increasing in the summer and decreasing in the winter, in correlation to body weight and fat mass. Furthermore, 24-h fasting decreases plasma leptin levels in young and mature mares (Fitzgerald and McManus, 2000). The present study showed that serum leptin concentration was higher than its cut-off point in the obese stallions in warmer months (Fig. 1). Our study is in accordance with Saad et al.'s study (1997) that showed that serum concentrations of leptin were higher in geldings and stallions versus mares, which differs from humans, in whom females have higher leptin concentrations. Leptin can be used to determine if a horse is in a positive or negative energy balance, providing data on body condition and percentage of fat mass. Buff et al. (2002) determined that serum leptin concentrations were positively correlated ( $r=0.64$ ;  $P<0.001$ ) with body condition score in a herd of 71 Quarter horses, which indicates that blood leptin concentrations reflect body fat mass in horses.

## Conclusion

This study comprehensively used multiple detailed parameters to scan thoroughly the concept of insulin resistance in Dare-

hshori horses on a long term basis. Based on our findings, this phenomenon was detected in aged and obese Darehshori stallions in warmer months. Furthermore, these findings represented insulin resistance phenomenon in a specific breed in Iran. The physiologic characteristics of this breed may be different from other ones and the conditions in Iran such as climate are different from other countries. Hence, it should be considered that Darehshori horses have unique characteristics that are different from other breeds. The study may be used as a guideline for evaluating the insulin resistance in Darehshori horses.

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## Conflicts of Interest

The author declared no conflict of interest.

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## مقاومت انسولینی در اسب های دره شوری و ارتباط آن با سن، جنس، فصل و چاقی

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### چکیده

**زمینه مطالعه:** سندرم متابولیک اسبان به عنوان یک اندوکراینوپاتی که نژاد های مختلف اسب ها و پونی ها را درگیر می کند در نظر گرفته شده است. این اختلال متابولیک، سندرمی چند عاملی است و به طور اولیه با چاقی، دیس لیپیدی و مقاومت انسولینی مرتبط است.

**هدف:** مطالعه حاضر به منظور ارزیابی پدیده مقاومت انسولینی در اسب های دره شوری در ارتباط با سن، جنس، فصل و چاقی انجام شد.

**روش کار:** ۱۲ نریان بالغ و ۱۴ مادبان بالغ نژاد دره شوری از دی ماه ۱۳۹۳ تا آبان ماه ۱۳۹۴ انتخاب شدند. ۱۴ اسب، کمتر و مابقی بیشتر از ۱۰ سال سن داشتند. نمره بدنی حیوانات بر اساس سیستم امتیازدهی ۱ تا ۹ هنکه تخمین زده شد که ۱ اسب لاغر و ۹ اسب بسیار چاق است. نمره بدنی ۱۲ اسب کمتر از ۵ و بقیه بیشتر از ۵ بود. اسب ها در خلال یک سال مورد مطالعه قرار گرفتند و هر ۴۵ روز خون گیری و ارزیابی وضعیت نمره بدنی انجام شد. پدیده مقاومت انسولینی بر اساس آزمون غربالگری ارزیابی شد. مقادیر سرمی گلوکز، انسولین و لپتین ارزیابی شدند.

نتایج: این مطالعه جامع، فراسنجه های متعددی را به منظور درک بهتر مقاومت انسولینی در اسب های دره شوری در یک بازه زمانی طولانی مدت مورد پایش قرار داد. مقادیر انسولین، گلوکز و لپتین در نریان های مسن و چاق در ماه های گرم تر به طور معنی داری بیشتر از سایرین و همچنین بیشتر از دامنه طبیعی بود ( $P < 0.05$ ). از این رو، در این گروه از اسب های مورد مطالعه، مقاومت انسولینی تشخیص داده شد.

**نتیجه گیری نهایی:** یافته های حاضر، پدیده مقاومت انسولینی را در یک نژاد خاص در ایران ارائه می دهد. ویژگی های فیزیولوژیک این نژاد ممکن است از سایرین متفاوت باشد و شرایط ایران نظیر وضعیت آب و هوا متفاوت از سایر کشورهاست. از این رو، باید در نظر داشت که اسب های دره شوری، خصوصیات منحصر به فرد متفاوتی از سایر نژادها دارند. این مطالعه، ممکن است به عنوان یک راهنما در ارزیابی مقاومت انسولینی در اسب های دره شوری مورد استفاده واقع شود.

### واژه های کلیدی:

مقاومت انسولینی، انسولین، گلوکز، لپتین، اسب دره شوری