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Possible dysmetabolic hyperferritinemia in hyperinsulinemic horses

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Abstract

Background: Hyperinsulinemia associated with equine metabolic syndrome and pituitary pars intermedia dysfunction is a risk factor for laminitis. Research in other species has shown elevated body iron levels as both a predictor and consequence of insulin resistance. In humans, this is known as dysmetabolic hyperferritinemia.

Aim: To explore the relationship between equine hyperinsulinemia and body iron levels.

Methods: We reviewed case histories and laboratory results from an open access database maintained by the Equine Cushing's and Insulin Resistance Group Inc. (ECIR). We identified 33 horses with confirmed hyperinsulinemia and laboratory results for serum iron, total iron binding capacity, and ferritin. Pearson correlation was used to test the relationship between insulin and iron indices. Additionally, we performed a secondary analysis of a previously reported controlled trial that was originally designed to test the correlation between iron status and the insulin response in horses. Here, we used a *t*-test to compare the mean values of insulin and ferritin between horses we categorized as normal or hyperinsulinemic based on their response to an oral challenge.

Results: Serum ferritin exceeded published reference range in 100% of the horses identified from the ECIR database. There were no statistically significant associations between insulin indices (RISQI, log insulin) and iron indices (log serum iron, log TSI%, log ferritin). There were trends for a negative association between RISQI and log iron [$r(31) = -0.33$, $p = 0.058$] and a positive association between age and ferritin [$r(30) = 0.34$, $p = 0.054$]. From the secondary data analysis of published data, we found significantly elevated ferritin ($p = 0.05$) in horses considered hyperinsulinemic by dynamic insulin testing compared to horses with a normal response.

Conclusion: These results suggest the potential for iron overload in hyperinsulinemic horses, a feature documented in other species and should stimulate further study into the relationship between insulin and iron dysregulation in the horse.

Keywords: Equine, Ferritin, Hyperinsulinemia, Iron overload.

Introduction

The relationship between iron regulation, glucose homeostasis, insulin action, and secretion is complex. High body burdens of iron have been associated with decreased insulin sensitivity in humans (Huang *et al*., 2015; Ryan *et al*., 2018; Zafar *et al*., 2015) and increased risk of developing insulin resistance/hyperinsulinemia and type 2 diabetes mellitus (Simcox and McClain, 2013) while reduction of iron levels via phlebotomy may improve metabolic parameters (Fargion *et al*., 2005; Houschyar *et al*., 2012). Similarly, several species of captive birds and mammals are known to develop dietary iron overload and subsequent insulin resistance on zoo diets, including the close equine relatives; the tapir and black rhinoceros (Clauss and Paglia, 2012).

Hyperinsulinemia is a component of equine metabolic syndrome (EMS) and may be a feature of pituitary pars intermedia dysfunction (PPID) (Frank and Tadros, 2014). Iatrogenic hyperinsulinemia while maintaining euglycemia induces laminitis in both ponies (Asplin *et al*., 2007) and horses (de Laat *et al*., 2010). Hyperinsulinemia is now believed to be by far the most common cause of naturally occurring laminitis (Morgan *et al*., 2015) and is a significant welfare issue with population studies showing 18% to 27% of horses/ponies being hyperinsulinemic (Durham *et al*., 2019).

Smith *et al.* (1984) published a ferritin normal range which was correlated to the iron levels in the liver and spleen. That work, including the development of species-specific assays, remains the gold standard today. Smith established that, as in other species, ferritin is the best index of the liver and spleen nonheme iron stores in horses, with a high correlation between serum ferritin and hepatic and splenic tissue levels.

Recognition of the link between iron and insulin resistance dates back to the early 1900s (Root, 1929). Since then, there has been considerable investigation of the role of iron in adipose tissue, effects of iron on glucose homeostasis, and the interplay between insulin and iron trafficking (Fernandez-Real *et al*., 2002) Suggested roles for insulin include stimulating enhanced uptake of extracellular iron and inducing relocation of transferrin receptors (Fernandez-Real *et al*., 2015). A genetic component is likely, suggested by significantly higher insulin and iron indices and a significant positive correlation between insulin and

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serum iron in the non-diabetic offspring of type 2 diabetics (Zafar *et al*., 2015).

While the relationship between iron and insulin resistance has been the topic of intense research investigation in humans and other species, it has been largely unrecognized by equine medicine. There are more reports describing the relationship between iron, inflammation, and insulin sensitivity in rhinos than in horses because investigators recognized the relationship between metabolic disease and iron overload in black rhinos (Schook *et al*., 2015). To the authors' knowledge, there is only a single controlled pilot study describing the relationship between iron status and the insulin response in horses (Nielsen *et al*., 2012). Nielsen *et al.* measured the insulin response to a glycemic challenge (dextrose or corn added to feed) and found a positive correlation between serum ferritin and insulin. There was also a conference presentation showing significantly elevated iron indices in hyperinsulinemic horses compared to herd mates but the herd mate controls, while phenotypically normal, were not formally tested for hyperinsulinemia (Kellon, 2006). Our goal in this short communication is to report ferritin levels in hyperinsulinemic horses in order to generate further investigation into equine insulin and iron dysregulation.

Materials and Methods

We performed a retrospective review of case histories and laboratory results; specifically, insulin and iron indices, obtained from a database maintained by the Equine Cushing's and Insulin Resistance Group Inc. (ECIR) *https://ecir.groups.io*. As members of the ECIR Group, horse owners provided detailed case histories and laboratory reports to the database. Blood samples were authorized by owners and collected by their attending veterinarian as part of the routine care and treatment of their animals.

We also performed a secondary analysis of previously reported ferritin levels in horses that had normal versus hyperinsulinemic responses to an oral challenge (Nielsen *et al*., 2012). Because Nielsen's dextrose challenge used a lower (0.25 g/kg BW) dose than recommended for the in-feed dextrose challenge (Durham *et al*., 2019), we used the results of the peak insulin response to pelleted-corn feeding (fed at 1.5 g/kg BW = 1.05 g/kg BW starch). This approach is justified by de Laat and Sillence (2017) who established equivalence between an oral dextrose load in a high fiber carrier and an equivalent amount of carbohydrate as grain.

Animals

ECIR database

We reviewed the case histories of privately owned horses of various breeds (20 geldings, 13 mares) ranging in age from 8 to 29 years (mean age 17.9 ± 5.8). Breed, age, sex, iron, and insulin indices for individual animals are listed in Table 1. None of the horses were reported to have factors which could influence ferritin levels (McLean *et al*., 1987; Smith *et al*., 1984), including active laminitis, injuries, evidence of other disease, pregnancy, or formal exercise within 48 hours prior to sampling (Hyyppä *et al*., 2002) and were not in regular work. Horses were on forage only or forage predominant diets and had no current supplemental iron intake or past history of supplemental iron other than what may have been present in commercial concentrates.

Nielsen study

Sixteen Arabian horses were included (eight geldings, eight mares) classified as young (age 3 years, $n = 9$) or older (>10 years, $n = 7$). Details of the feeding protocol, sample collection, and testing from Nielsen *et al*. (2012) are found in the original publication.

Insulin and iron indices

All samples were drawn and processed by owners' private attending veterinarians. Blood for serum iron, total iron binding capacity (TIBC), and ferritin was processed according to instructions from the Comparative Hematology Laboratory, Kansas State University (KSU) including avoiding hemolysis while collecting serum samples, removing serum from clot into a capped plastic tube, and freezing at -20°C. Samples were shipped to KSU overnight with ice gel packs or dry ice. Testing for ferritin was performed as detailed by the laboratory founder (Smith *et al*., 1984).

Blood for insulin determination was drawn in the non-fasted state with horses having access only to soaked hay or hay with combined ethanol soluble carbohydrate and starch of ≤10% for 12 or more hours prior to sampling. Venous samples were collected into EDTA or serum tubes, processed, and shipped according to sample handling instructions from the Animal Health Diagnostic Laboratory at Cornell University School of Veterinary Medicine and analyzed via validated radioimmunoassay.

The proxy for insulin sensitivity [reciprocal of the square root of insulin: (RISQI)] was calculated from basal plasma insulin as follows: RISQI = Insulin$^{-0.5}$(=1/√ insulin). Transferrin saturation index (TSI) as a marker of possible disease/inflammatory effects on iron homeostasis and ferritin was calculated from serum iron and TIBC by the equation Iron/TIBC × 100.

For the retrospective analysis of Nielsen *et al*. (2012), we used current guidelines to define hyperinsulinemia (Durham *et al*., 2019). We grouped animals based on the published report of their response to the pelleted corn feeding challenge; HYP (hyperinsulinemia) and NORM (normoinsulinemia).

Statistical analysis

ECIR sample

Insulin and iron indices obtained from the ECIR database were tested for normality using the Shapiro–Wilk test. Data with abnormal distributions (serum iron, TSI%, ferritin, and insulin) were log-transformed to achieve a normal distribution; RISQI was normally

Table 1. Insulin and iron data of horses from the ECIR database.

Breed	Age (years)	Sex	Iron (µg/dl)	TSI%	Ferritin (ng/ml)	Insulin (µIU/ml)	RISQI (uIU/l)$^{-0.5}$
Friesian	9	Mare	176	50.4	620	259.0	0.06
Arabian	15	Gelded	277	49.1	-	168.5	0.08
Arabian	19	Gelded	220	47.1	430	55.8	0.13
Quarterhorse	17	Gelded	214	38.4	855	38.7	0.16
Warmblood/Arabian	16	Gelded	158	40.4	694	26.7	0.19
Arabian	19	Gelded	213	43.3	449	47.5	0.15
Morgan	13	Mare	231	50.9	373	101.0	0.10
Tennessee Walker	14	Gelded	224	44.3	308	107.5	0.09
Appaloosa	15	Gelded	194	48.7	980	104.0	0.09
Saddlebred	8	Gelded	170	41.4	340	51.7	0.14
Kentucky Mountain	13	Gelded	190	47.9	268	125.0	0.09
Warmblood	16	Gelded	201	57.8	479	47.7	0.14
Arabian	18	Gelded	154	57.5	430	55.9	0.13
Miniature	24	Mare	345	61.7	458	121.7	0.09
Morgan	20	Gelded	352	69.4	261	22.0	0.21
Gypsy Vanner	11	Gelded	157	39.6	381	31.2	0.18
DraftXTb	21	Gelded	141	44.9	656	27.9	0.19
ShireXPinto	21	Mare	125	44.2	490	41.8	0.14
Morgan	22	Gelded	308	58.1	308	98.6	0.10
Rocky Mountain	18	Gelded	312	70.4	370	24.0	0.20
Thoroughbred	27	Mare	137	37.4	758	23.0	0.21
Haflinger	9	Mare	318	92.4	266	45.8	0.15
Rocky Mountain	15	Mare	152	44.9	356	52.0	0.14
Arabian	12	Mare	140	41.2	317	47.0	0.15
Arabian	25	Gelded	157	73.3	435	79.0	0.11
Warmblood	17	Mare	160	45.3	324	36.5	0.17
Warmblood	18	Mare	121	33.4	542	27.0	0.19
Warmblood	10	Mare	269	65.1	479	42.4	0.15
Spotted Saddle	29	Gelded	289	74.1	722	109.2	0.09
Quarterhorse	18	Gelded	214	38.5	844	38.7	0.16
Thoroughbred	27	Mare	137	34	758	21.0	0.22
Morgan	27	Mare	168	34	271	78.7	0.11
Warmblood	27	Gelded	307	54.9	982	208.0	0.07

distributed. We used Pearson correlation to test the relationship between iron and insulin indices and age.

Nielsen sample

Data from Nielsen *et al*. (2012) were similarly tested for normality. Ferritin was normally distributed, and peak insulin to pelleted-corn was log-transformed to a normal distribution. For these data, we compared independent means of log peak insulin and ferritin between groups NORM and HYP using an unpaired *t*-test. Results were considered significant at $p \leq 0.05$, and trends reported at $p \leq 0.1$.

Results and Discussion

ECIR sample

Mean ± SD for normally distributed data (age, RISQI) and median (IQR) for log-transformed data (serum iron, TIBC, and ferritin), as well as reference values, are listed in Table 2. All animals were confirmed hyperinsulinemic by basal insulin (>20 uIU/ml) (Durham *et al*., 2019) and screened within or below the lowest quintile of insulin sensitivity by RISQI (0.159 uIU/l$^{-0.5}$) (Treiber *et al*., 2005). One horse was

Table 2. Mean insulin level and iron indices in ECIR horses with reference ranges.

Parameters	Mean ± SD; Median (IQR)	Laboratory or published reference range
Age (years)	17.9 ± 5.8	
Insulin (uIU/ml)	47.7 (66.6)	<20 uIU/ml; (Frank and Tadros, 2014)
RISQI	0.14 ± 0.05	>0.32; (Treiber *et al*., 2005)
Iron (ug/dl)	194 (115)	50–198 ug/dl (Smith *et al*., 1984)
TSI%	47.1 (16.9)	30%–40% (McLean *et al*., 1987)
Ferritin (ng/ml)	442 (343)	43–261 ug/dl (Smith *et al*., 1984)

missing results for ferritin. In the 32 animals with results for ferritin, 100% exceeded the published normal range. Results of the Pearson correlation indicated no statistically significant associations between insulin indices (RISQI, log insulin) and iron indices (log serum iron, log TSI%, and log ferritin). We found trends for a negative association between RISQI and log iron [$r(31) = -0.33$, $p = 0.058$] and a positive association between age and ferritin [$r(30) = 0.34$, $p = 0.054$].

Nielsen sample

The peak insulin response to pelleted corn in 6 of the 16 horses was abnormally high and a 7th horse was only 1 uIU/ml below the >85 uIU/ml threshold. These seven animals made up the HYP group. The remaining nine animals with typical responses to the corn feeding challenge made up the NORM group. Log transformed peak insulin to corn feeding was significantly higher in HYP horses ($t = 5.40$, $p = < 0.0001$); raw data; 247 uIU/ml ± 207 compared to 40.9 uIU/ml ± 13.7 for NORM horses (Fig. 1). Similarly, ferritin was significantly higher in the HYP horses 556.3 ± 178.3 compared to 374.6 ± 165.0 in NORM horses; ($t = -2.11$, $p = 0.05$) (Fig. 1). We should point out that Nielsen *et al*. (2012) found a significant correlation between serum ferritin and insulin response to dextrose or pelleted corn feeding; however, the significance of the correlation was largely driven by one highly hyperinsulinemic horse. We approached the data differently by grouping the horses into hyperinsulinemic vs. normal in response to corn feeding, correcting the skewed insulin data distribution by log-transformation, and testing the difference in group means.

All horses from the ECIR database fulfilled the definition of EMS by being hyperinsulinemic at baseline plus one or more of the characteristics of generalized or regional adiposity, weight loss resistance, hyperlipidemia,

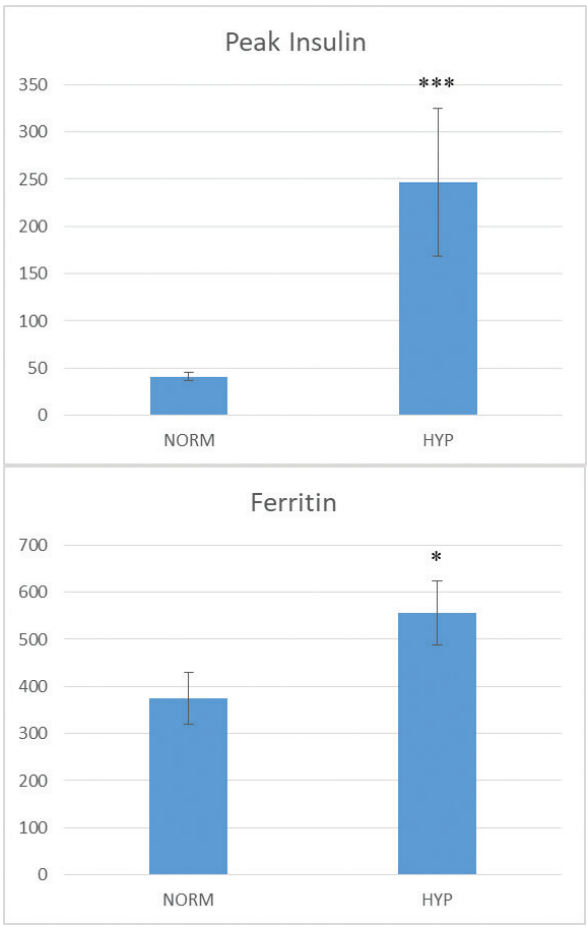

Fig. 1. Raw (non-transformed) data from Nielsen *et al*. (2012). Mean (+/-) SE for peak insulin to pelleted corn feeding (top) and ferritin (bottom) after grouping animals as normoinsulinemic (NORM) versus hyperinsulinemic (HYP) (***p =< 0.0001, *p = 0.05).

and laminitis history. Tissue insulin resistance per se was not confirmed by intravenous testing. However, RISQI values fell within or below the lowest quintile of insulin sensitivity as determined by Treiber *et al*. (2005) using FSIGT to validate the proxies. RISQI has been used as a surrogate for intravenous testing in other published studies (Turner *et al*., 2011). Sensitivity of the insulin resistance proxies is known to be poor but the specificity of RISQI is 85%. Decreased hepatic/tissue insulin clearance has been established as a factor in hyperinsulinemia but it has not been shown that hyperinsulinemia caused by reduced hepatic/tissue metabolism of insulin can occur outside of the insulin-resistant state or as a prodrome. Therefore, while it may be a factor in addition to tissue insulin resistance which contributes to the hyperinsulinemia, it does not necessarily occur independently of it.

All horses in our ECIR retrospective analysis had hyperinsulinemia and elevated ferritin. We also

found a trend for lower RISQI (higher insulin) with higher serum iron, which may reflect increased iron absorption. Mice placed on a diet designed to induce insulin resistance were found to show increased hepatic iron and decreased hepcidin expression prior to the development of insulin resistance, suggesting increased iron absorption is an integral and early event (Tsuchiya *et al*., 2013).

The lack of a significant linear relationship between insulin and ferritin was not unexpected given that both iron overload and hyperinsulinemia are multifactorial states. The degree of hyperinsulinemia may be influenced by such factors as genetics, diet, exercise, and PPID. Although care was taken to exclude animals with current factors known to influence ferritin, age, and varying iron levels in forage and water over the life of the animal could contribute to the degree of iron overload, as would the existence of dietary factors interfering with iron absorption.

We found a trend for higher ferritin with advancing age in this small sample, a possibility suggested by others (Smith *et al*., 1984). Since there is no avenue of excretion for excess iron, the degree of iron overload will represent iron accumulation over time and also may be more representative of the duration of hyperinsulinemia rather than hyperinsulinemia per se or degree of hyperinsulinemia.

Considerable interplay has been described between iron metabolism and metabolic syndrome and type 2 diabetes in humans (Kunutsor *et al*., 2013), laboratory animals (Wang *et al*., 2015), and zoo animals (Clauss and Paglia, 2012). Increased body iron stores are a risk factor for insulin resistance and the development of type 2 diabetes (Kunutsor *et al*., 2013). Reduction of iron via phlebotomy may improve insulin sensitivity in humans (Fernandez-Real *et al*., 2002) while a low heme iron diet reduces the iron status and risk (Hua *et al*., 2001). Research in insulin-resistant, type 2 diabetic rats have demonstrated increased intestinal absorption of iron (Weeks *et al*., 1989), making the iron-insulin link a self-perpetuating situation. Our data suggest similar interactions between hyperinsulinemia and body iron burden that could exist in the horse and are directions for future study.

Many factors other than hyperinsulinemia may be associated with altered iron status. Serum ferritin is also an acute phase reactant. Therefore, exercise, illness, and injury are known to increase serum ferritin levels. Pregnancy reduces ferritin levels to guarantee adequate iron availability for the fetus. Care was taken to exclude horses with a history of these possible confounders. The majority (79%) of horses in our sample had high TSI while none were low. Low TSI is associated with inflammation (McLean *et al*., 1987; Rambod *et al*., 2008). The observation of normal to elevated TSI in the sample where all horses had elevated ferritin is further evidence of no significant inflammatory component in this cohort, compatible with the elevated ferritins indicating iron overload.

We recognize limitations to this retrospective analysis. This was not a prospective, controlled study comparing age-matched groups with and without hyperinsulinemia. The existing evidence investigating the bidirectional relationship between iron and hyperinsulinemia in horses is extraordinarily limited despite considerable scientific inquiry in other species. To the authors' knowledge, Nielsen *et al*. (2012) is the only controlled study that used an equine model, but as a proxy for captive rhinos. A controlled, prospective study of sufficient power using dynamic tests of insulin sensitivity, diet control and analysis, iron indices, and inflammatory markers or other factors influencing ferritin is needed to disentangle the complex relationship between insulin and iron in the horse.

The term for the relationship between body iron stores and insulin in humans is dysmetabolic hyperferritinemia (Stechemesser *et al*., 2017). The influence is bidirectional, with iron burden influencing insulin levels and vice versa. Iron has been suggested as a therapeutic target for people with, or at risk of, hyperinsulinemia and type 2 diabetes (Fernandez-Real *et al*., 2015) as well as laboratory animal models (Minamiyama *et al*., 2010), Macaws (Gancz *et al*., 2007), dolphins (Venn-Watson *et al*., 2013), and other zoo species. If the situation is confirmed to be similar in horses, iron may be a modifiable risk factor for hyperinsulinemia.

While even severe iron overload can occur in horses without evidence of metabolic syndrome (Theelen *et al*., 2019), and iron is unlikely to be an independent causal factor, the interplay uncovered in other species shows this is a factor worthy of further investigation. This preliminary retrospective investigation should increase awareness of the potential for iron overload in hyperinsulinemic horses and stimulate further study into the relationship between insulin and iron dysregulation.

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Conflicts of interest

The authors declare that there are no conflicts of interest.

Author contributions

E.K.: concept/design, acquisition of data, drafting of the manuscript, critical revision of the manuscript, and approval of the manuscript. K.G: preparation of figures, statistical analysis, drafting of the manuscript, critical revision of the manuscript, and approval of the manuscript.

Ethical approval

Not necessary—a retrospective review of case histories and published data.

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