Relationship between intracellular free magnesium concentration and the degree of insulin resistance in horses with equine metabolic syndrome

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Summary: Magnesium metabolism, especially the intracellular magnesium concentration $[Mg^{2+}]_i$, plays an important role in human diabetes mellitus. The aim of this study was to evaluate $[Mg^{2+}]_i$ in horses with insulin resistance and to examine a possible association with other markers of insulin dysregulation. The study included 38 horses with a positive combined glucose insulin tolerance test (CGIT) and phenotypic signs of EMS. Baseline glucose (G_0), glucose curve, baseline insulin (I_0), insulin after 45 minutes (I_{45}), total serum magnesium concentration and $[Mg^{2+}]_i$ were measured. The reciprocal inverse square of insulin (RISQI), the modified insulin-to-glucose ratio (MIRG) and the insulin difference (I_{45-0}) were calculated. A t-test for independent samples was used to compare $[Mg^{2+}]_i$ in healthy and EMS horses. Linear regression analyses with uni- and multivariable models were calculated to evaluate the influence of $[Mg^{2+}]_i$ on all other blood and clinical parameters. Compared to healthy controls, $[Mg^{2+}]_i$ was significantly lower in horses with EMS (P = 0.015). Univariable linear regression models showed significant associations between I_0 (P = 0.004, b = 0.006), I_{45} (P = 0.006, b = 0.001), I_{45-0} (P = 0.008, b = 0.001), RISQI (P = 0.009, b = –0.158), MIRG (P = 0.001, b = 0.017) and $[Mg^{2+}]_i$. The multivariable model revealed that MIRG significantly influenced $[Mg^{2+}]_i$ whereas the other factors had no additional influence. Horses with EMS exhibited an intracellular magnesium deficit that can be partially reversed by increased secretion of insulin. These findings substantiate the crucial role for insulin in regulating $[Mg^{2+}]_i$ and its relevance in EMS.

Keywords: magnesium, lymphocytes, insulin dysregulation, diabetes mellitus

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Introduction

Diabetes mellitus is a worldwide problem as is the equivalent equine metabolic syndrome (EMS). Key features of both diseases are insulin dysregulation (ID), chronic inflammatory state and dysfunction of the adipose tissue (*Frank* et al. 2010). Human diabetes mellitus type II patients often display a magnesium deficit in the serum or at the intracellular level and supplementation with magnesium can improve the insulin sensitivity (*Volpe* 2008, *Günther* 2010). Multiple reasons for magnesium deficits have been suggested in humans. Diabetes patients are more likely to consume an unhealthy diet with a low magnesium content (*Paolisso* and *Barbagallo* 1997). Additionally, losses of magnesium in the urine due to increased tubular flow and reduced tubular absorption in the kidney (hyperglycemic glycosuria) are possible (*Ponder* et al. 1990, *Pham* et al. 2007). Magnesium influences the tyrosine kinase at the insulin receptor and leads to an increased uptake of glucose into the tissue. Insulin, on the other hand, can induce a shift of magnesium from the extracellular fluid into the cell (*Mastrototaro* et al. 2015). Furthermore, under stress conditions (such as decreased insulin sensitivity), activation of various transporters like the magnesium transporter "solute carrier family 41, member A1" (SLC41A1) may result in active magnesium transport out of the cell. Insulin resistance may thus promote an intracellular magnesium deficiency (*Mastrototaro* et al. 2015, *Romani* and *Maguire* 2002) and this, in turn, could exacerbate the insulin resistance. Finally, magnesium deficiency could lead to a mismatch of calcium and magnesium. The latter results in increased vascular tone and increased secretion of catecholamines by sympathetic nerve endings (*Itani* et al. 2000). Ultimately, these changes trigger hypertension in humans and could play a role in equine laminitis as well.

Horses' diets typically contain high amounts of magnesium and baseline hyperglycemia with glycosuria and urinary losses of magnesium rarely occur (*Stewart* et al. 2004). A whole body magnesium deficit is therefore unlikely to develop. However, shifts from the intracellular to the extracellular level are reasonable, resulting in an intracellular magnesium deficit.

The serum magnesium content contributes only 0.3% to the total body magnesium content. When the serum concentration is too low, shifts from the intracellular compartment to the serum keep the serum magnesium concentration within the reference range (*Vormann* 2003, *Arnaud* 2008). The serum magnesium concentration is therefore an insensitive method to evaluate the tissue magnesium concentration (*Chaudhary* et al. 2010). Tissue biopsies would be a more precise, but also more invasive procedure. The intracellular magnesium concentration $[Mg^{2+}]_i$ in blood lymphocytes (*Princi* et al. 1997, *Delva* et al. 2006), erythrocytes (*Basso* et al. 2000) or platelets (*Niemela* et al. 1997, *Yoshimura* et al. 1998) is similar to the concentration in the tissue. Therefore it has been used as a preferred marker of the magnesium metabolism (*Princi* et al. 1997). Methods to determine blood cell $[Mg^{2+}]_i$ (*Reinhart* et al. 1987, *Schwinger* et al. 1987) have been used in human medicine since the 1980s and reference values for $[Mg^{2+}]_i$ in healthy horses have previously been established (*Winter* et al. 2018).

Measurements of $[Mg^{2+}]_i$ in horses with ID could give new insights into a possibly altered magnesium metabolism and present a new diagnostic and research tool. Therefore, the aim of this study was to evaluate $[Mg^{2+}]_i$ in horses with insulin resistance and to examine a possible association with other markers of insulin dysregulation. Proceeding from results in humans, we hypothesized that $[Mg^{2+}]_i$ is inversely correlated with parameters of insulin resistance in EMS horses.

Material and Methods

Horses

In this study, 38 horses with different degrees of ID were examined. The horses were client-owned patients that were admitted to our clinic with an EMS phenotype between July 2016 and October 2017.

Criteria for inclusion were as follows: normal findings on clinical examination except for general and/or regional obesity; body condition score (BCS) ≥ 7; cresty neck score (CNS) ≥ 3; ≥ 4 years of age; negative test results for pituitary pars intermedia dysfunction (normal plasma ACTH concentration); no acute laminitis; and no magnesium supplementation during the last three months. All subjects had to show insulin dysregulation in the combined glucose insulin tolerance test (CGIT) (prolonged time (≥ 90 minutes) of the glucose curve to drop to baseline and/or increased baseline insulin (I_0; $> 20 \mu U/mL$) and/or increased insulin after 45 minutes (I_{45}; $> 100 \mu U/mL$)).

Experimental design

After admission, body weight, height, CNS (*Carter* et al. 2009) and BCS (*Henneke* et al. 1983) were measured. Horses were acclimatized for at least 12 hours and fasted for another 6 hours. Blood sampling and the CGIT were performed the morning after arrival. Blood samples to determine triglycerides (TG), gamma-glutamyl transferase (GGT), fructosamine and the total serum and $[Mg^{2+}]_i$ were taken immediately before the start of the CGIT. A clinical and orthopedic examination

(including laminitis grading according to *Obel* 1948) were performed after completion of the CGIT.

CGIT

The evening before testing, an IV catheter (Infusion catheter Set G14, Walter Veterinär-Instrumente e.k., Baruth/Mark, Germany) was inserted into one of the jugular veins under local and antiseptical conditions. To determine baseline glucose (G_0) and insulin (I_0) concentrations, blood samples were collected from the jugular vein catheter immediately before the start of the CGIT into a blood collection syringe containing lithium heparin (BD A-Line™, Arterial Blood Collection Syringe, Beckton, Dickinson and Company, Plymouth, United Kingdom) and tubes containing microscopic silica particles (EDTA tubes 4 ml and Lithium heparin tubes 10 ml, Sarstedt AG & Co, Nümbrecht, Germany). The CGIT procedure was conducted as described previously (*Eiler* et al. 2005). Briefly said, 150 mg/kg glucose (Glucose 40% ad us. vet., B. Braun Melsungen AG, Melsungen, Germany) and 0.1 mg/kg porcine insulin (Caninsulin®, Intervet Deutschland GmbH, Unterschleißheim, Germany) were given IV. Glucose concentrations were measured after 1, 5, 15, 25, 35, 45, 60, 75, 90, 105, 120, 135 and 150 minutes. Additionally, insulin was measured at 45 minutes (I_{45}). G_0 and I_0 concentrations were used to calculate the proxies reciprocal inverse square of insulin (RISQI), a parameter for insulin sensitivity, modified insulin-to-glucose ratio (MIRG), a parameter for β-cell function (*Treiber* et al. 2005) and the homeostasis model assessment for insulin resistance (*Fukushima* et al. 1999, *Kronfeld* et al. 2005) index (HOMA-IR).

Intracellular magnesium concentration

Blood samples for the measurements of $[Mg^{2+}]_i$ were collected into tubes containing lithium heparin (Lithiumheparin tubes 10 ml, Sarstedt AG & Co, Nümbrecht, Germany). The samples were processed immediately to isolate equine blood lymphocytes and measure $[Mg^{2+}]_i$ by spectrophotometry (LS 55 spectrophotometer, PerkinElmer, Rodgau, Germany) (*Winter* et al. 2018).

Analysis of blood samples

Plasma glucose concentrations were measured photometrically with an automated clinical blood gas analyzer (cobas b 123, Roche Deutschland Holding GmbH, Berlin). GGT and TG were measured by photometry with an automated clinical chemistry analyzer (Reflotron® plus Roche Deutschland Holding GmbH, Grenzach-Wyhlen/Germany). Serum insulin, serum fructosamine and total serum magnesium concentrations were analyzed in an external laboratory (Laboklin GmbH & Co. KG, Bad Kissingen, Germany), certified according to DIN EN ISO/IEC 17025:2005. Plasma insulin concentrations were determined by chemiluminescence (*Köller* et al. 2016) and serum fructosamine as well as total serum magnesium concentrations by photometry.

Statistics

All statistical analyses were performed with the statistical program IBM SPPS (version 24). P-values < 0.05 were considered

as statistically significant for all analyses. All data are presented as mean ± standard deviation (SD) except for non-normally distributed data such as CNS and BCS, which are presented as median and interquartile range. A t-test for independent samples was used to compare $[Mg^{2+}]_i$ in healthy and insulin-dysregulated horses and between sexes. Linear regression analyses with uni- and multivariable models were calculated to evaluate the influence of blood and clinical parameters on $[Mg^{2+}]_i$. All parameters that were statistically significant in univariable models were included in a multivariable model. From this model, non-significant variables were removed stepwise according to the change of the adjusted R^2 until only significant variables were left. Model diagnostics included test for normality of residuals and visual test of homoscedasticity.

Results

Horses

The study included 38 horses with a mean age of 14 ± 5 years. Five of the horses were Warmbloods, 21 were ponies and 12 were different other breeds (cold-blooded horse (n = 3), Standardbred (n = 2), Arabian horse (n = 2), Pura Raza Española (n = 2), Lusitano (n = 1), Appaloosa (n = 1) and Quarter Horse (n = 1)). Of the horses, 60.5 % (23/38) were mares, 39.5 % (15/38) geldings. They had a mean body weight of 419 ± 148 kg, a median CNS of 4 (IQR 3.75–5) and a median BCS of 8 (IGR 7–9). Signs of acute laminitis at admission were present in 7.9 % (3/38). These horses were tested for EMS before discharge, after the acute laminitis phase was over. A history of laminitis without clinical signs at admission was present in 18.4 % (7/38) of horses. The majority of horses (86.8 % (33/38)) were admitted because the owner or treating veterinarian had recommended an EMS test, most commonly due to reduced performance. Most horses were used as leisure horses, trained two to three times per week for up to one hour and had unlimited access to hay. Of all EMS horses, 26.3 % (10/38) were kept on pasture and 50 % (19/38) received grains or additional sweet feed. According to the owners, none of the horses showed muscle tensions, nervous temper, reduced stress resistance or recurrent signs of colic. At the time of the EMS test, 97.4 % (37/38) of horses had an Obel Score of 0, 2.6 % (1/38) a score of 1.

Blood values and CGIT

Total serum magnesium concentrations were within the reference range (0.5–0.9 mmol/L) in all horses. TG and GGT were slightly increased in 5.3 % (2/38) of horses respectively. The fructosamine concentration was increased in 5.3 % (2/38) with a mean concentration of 314.9 ± 29.8 µmol/L. G_0 was increased in 7.9 % (3/38) of horses with a mean concentration of 5.13 ± 0.63 mmol/L (92.4 ± 11.3 mg/dL). I_0 was within the reference range in all horses with a mean concentration of 7.5 ± 4.3 µU/mL. I_{45} was increased in 18.4 % (7/38) with a mean concentration of 71.8 ± 43.1 µU/mL. RISQI was decreased in 23.7 % (9/38) with a mean value of 0.42 ± 0.16 (mU/L)$^{0.5}$ and MIRG was increased in 21.1 % (8/38) with a mean value of 4.4 ± 2.1 mU^2/10×L×mg. Both RISQI and MIRG were not within the reference range in 15.8 % (6/38)

of horses. Glucose concentrations were decreased back to baseline in a mean time of 143 ± 40 minutes.

HOMA index (*Fukushima* et al. 1999) was categorized as stage 1 (HOMA < 2; an insulin resistance is unlikely) in 68.4 % (26/38) of horses, as stage 2 (HOMA 2.0–2.5; an indication of insulin resistance is given) in 10.5 % (4/38) and as stage 3 (HOMA 2.5–5.0; an insulin resistance is most likely) in 21.1 % (8/38) of horses. None of the horses was in stage 4 (HOMA > 5; an insulin resistance in present).

Intracellular magnesium concentration and correlation with insulin dysregulation

$[Mg^{2+}]_i$ was within the reference range in 92.1 % (35/38), below the reference ranges in two horses and above the reference ranges in one horse. $[Mg^{2+}]_i$ was significantly lower in EMS horses (0.24 ± 0.074 mmol/L) than in the healthy controls (0.29 ± 0.067 mmol/L; P = 0.015). $[Mg^{2+}]_i$ was not significantly different between sexes (P = 0.075).

Linear regression analyses with univariable models showed significant associations between $[Mg^{2+}]_i$ and each of the variables I_0 (P = 0.004, b = 0.006), I_{45} (P = 0.006, b = 0.001), I_{45-0} (P = 0.008, b = 0.001), RISQI (P = 0.009, b = –0.158) and MIRG (P = 0.001, b = 0.017). The final multivariable model showed a significant influence of MIRG (P = 0.001, b = 0.017) on $[Mg^{2+}]_i$. All other variables turned out to be not significant after considering the effect of MIRG. Thus, the $[Mg^{2+}]_i$ can be predicted most accurately in EMS horses from MIRG where MIRG increases by 1 represent $[Mg^{2+}]_i$ increases by 0.017 mmol/L (Figure 1).

Discussion

In this study, horses with equine metabolic syndrome had $[Mg^{2+}]_i$ values that were by and large within the previously established reference range. However, $[Mg^{2+}]_i$ values were generally lower in horses with EMS than in healthy control horses.
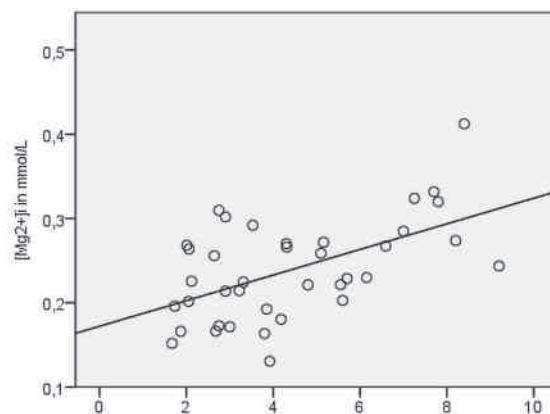

Fig. 1   Scatter plot displaying the linear relationship between the modified insulin-to-glucose ratio (MIRG) in mU^2/10×L×mg and the intracellular magnesium concentration $[Mg^{2+}]_i$ in mmol/L in horses with EMS. | *Streudiagramm, das die lineare Beziehung zwischen dem modifizierten Insulin-zu-Glucose-Verhältnis (MIRG) in mU^2/10×L×mg und der intrazellulären Magnesiumkonzentration $[Mg^{2+}]_i$ in mmol/L bei Pferden mit EMS zeigt.*

The second important finding was a positive correlation between $[Mg^{2+}]_i$ and β-cell function within this group of EMS horses. Regression analysis revealed that if MIRG increased by 1, $[Mg^{2+}]_i$ increased by 0.017 mmol/L. This means that EMS horses with a higher insulin secretion from β-cells have higher $[Mg^{2+}]_i$.

In human medicine, especially in the pathophysiology of diabetes mellitus type II, the role of magnesium is well defined. An inverse relationship between serum magnesium concentration and the incidence of diabetes mellitus type 2 was ascertained. Several studies showed that up to 77% of type 2 diabetics are affected by magnesium deficiency (*Pham* et al. 2007, *Sales* et al. 2011, *Rasheed* et al. 2012). Moreover, the supplementation with magnesium reduces the risk to develop diabetes in humans (*Rasheed* et al. 2012, *Yang* et al. 1999, *He* et al. 2006). Magnesium supplementation has a positive influence on different parameters related to the control of blood glucose concentration and counteracts the development of an insulin resistance (*Volpe* 2008, *Günther* 2010, *Hruby* et al. 2017). Interestingly, previous studies in humans have shown, that inducing a magnesium deficiency can reduce insulin sensitivity; whereas, a four week magnesium supplementation improved glucose handling in elderly people without diabetes (*Paolisso* et al. 1992, *Nadler* et al. 1993). This suggests an influence of magnesium on glucose metabolism irrespectively of whether the test persons already have clinical diabetes.

In EMS, research on the role of magnesium has been very scarce and not that consistent. The parallels between EMS and diabetes mellitus type II could suggest a similar role for magnesium in both diseases. In the present study, we discovered a significantly lower $[Mg^{2+}]_i$ in EMS horses identified by decreased insulin sensitivity, but values were still within the previously established reference range (*Winter* et al. 2018). One possible explanation for this relatively small difference in $[Mg^{2+}]_i$ could be the comparatively high magnesium intake with the typical equine diet. It is well established in humans, that oral magnesium supplementation can counteract the development of intracellular magnesium deficiency (*Thomas* et al. 2000). A second possible explanation for the relatively small drop in $[Mg^{2+}]_i$ in EMS patients might be related to the severity of ID. In human medicine, a distinction is made between impaired glucose tolerance (IGT), impaired fasting glycaemia (IFG), also called "non-diabetic fasting hyperglycemia", and diabetes mellitus with hyperglycemia (*Alberti* and *Zimmet* 1998). In the above-mentioned studies on the relationship between magnesium and ID in humans (*Paolisso* and *Barbagallo* 1997, *Pham* et al. 2007, *Chaudhary* et al. 2010, *Rasheed* et al. 2012, *Kao* et al. 1999, *de Valk* 1999) patients were classified as diabetes mellitus type II. Hence, these patients showed hyperglycemia at rest and a more pronounced ID than the horses in our study. Glucose baseline concentrations were within the reference range in 92.1% (35/38) of our horses and all showed physiological baseline concentrations of insulin. In addition, factors that contribute to magnesium deficiency in humans (renal wasting and reduced intake) are not relevant in horses. Perhaps a more severe ID or insulin resistance would be associated with a more distinct magnesium deficiency. In addition to altered glucose and insulin levels, laminitis is an important consequence of pronounced ID. Only 7.9% (3/38) of the horses showed acute laminitis when admitted to our clinic and were examined for our study before discharge. A history of laminitis was present in only 18.4% (7/38) of the horses.

The theory that a rather mild ID can be made responsible for the small decreases in $[Mg^{2+}]_i$ in the EMS horses of our study is supported by the finding that the decreased $[Mg^{2+}]_i$ in EMS horses was partially reversed by increased secretion of insulin from pancreatic β-cells. The latter can be postulated because horses with higher MIRG suffered less from lower $[Mg^{2+}]_i$. A higher MIRG, in turn, is an indicator of the ability of the pancreas to alleviate the consequences of insulin resistance by increased secretion of insulin. The increased secretion of insulin may subsequently rescue the insulin signaling pathway, leading to an increased activation of the phosphoinositide 3-kinase (PI3K) by the insulin receptor. PI3K then further activates protein kinase B (PKB), which induces a higher activity of the phosphodiesterase. As a result, the cAMP concentration decreases and the protein kinase A (PKA) is less active. Less active PKA results in less phosphorylation of the Na^+/Mg^{2+} exchanger SLC41A1 and, as an end result, in less efflux of Mg^{2+} out of the cell (*Mastrototaro* et al. 2015, *Zmuda-Trzebiatowska* et al. 2006, *Ahmed* et al. 2010). Via this pathway, insulin application is able to retain magnesium in the intracellular compartment in non-diabetic humans with a decline in plasma and an increase in intracellular magnesium concentration (*Paolisso* et al. 1986). Vice versa, inhibition of SLC41A1 by insulin becomes ineffective in decompensated insulin resistance with increased glucose baseline concentration, provoking an intracellular magnesium deficit.

Similar to diabetes, supplementation with magnesium has also been investigated as a treatment option in horses with ID with varying success. Depending on the source, the daily requirement for elemental magnesium in adult horses is between 12.5–20 mg/kg BW/day. For high performance horses (pregnancy, lactation, sports), demand can increase to as much as 30 mg/kg BW/day (*Hintz* et al. 1973, *Horses NRCCoNRo* 2007). For oral supplementation of horses, the following dosages are recommended: 30–50 mg/kg of magnesium oxide BW/day, 80–100 mg/kg of magnesium sulfate BW/day or 125–300 mg/kg of magnesium-aspartate hydrochloride BW/day (*Toribio* 2010). In one study by *Chameroy* et al., the effects of a feed additive containing chromium and 8.8 g magnesium oxide/proteinate per horse on insulin sensitivity have been investigated. No differences were found in the blood levels of glucose and insulin immunoreactivity or in the insulin sensitivity of peripheral tissues (*Chameroy* et al. 2011). Another study showed improvements in the CGIT (shorter positive phase duration of the glucose curve) in 3/5 horses and a trend for a lower fructosamine concentration after three months of supplementation with 30 mg/kg magnesium as magnesium-aspartate hydrochloride (*Winter* et al. 2016). Because of the small sample size and limited repeatability of the glucose curve, results of the latter study should be interpreted with care. It is possible that horses with ID have an increased need of magnesium and a dosage above the usual requirements is necessary to induce changes in insulin sensitivity. Furthermore, studies in cats and rats have shown that magnesium-aspartate hydrochloride is significantly better absorbed from the gastrointestinal tract than magnesium sulfate, chloride or aspartate (*Classen* et al. 1973). In addition, magnesium-aspartate hydrochloride has

a better oral bioavailability than magnesium oxide in humans (*Mühlbauer* et al. 1991). Comparable examinations in horses have not been carried out so far. Therefore, differences in study design may explain varying results.

The results of the present study confirm earlier findings from human medicine in that serum concentrations of magnesium are an insensitive marker of magnesium deficiency. Therefore, different methods for the determination of $[Mg^{2+}]_i$ in multiple tissues and blood cells have been developed (*Reinhart* et al. 1987, *Schwinger* et al. 1987, *Huijgen* et al. 1998). Values obtained by these techniques in skeletal (*Princi* et al. 1997), vascular smooth muscle, cardiac muscle (*Niemela* et al. 1997), lymphocytes and erythrocytes (*Princi* et al. 1997, *Delva* et al. 2006, *Basso* et al. 2000) reflect the actual situation in the cell better than the serum magnesium concentration.

Different tests have been described in literature to diagnose ID in horses. The CGIT, which was used in this study, is easy to perform and a commonly used test. In comparison, the frequently sampled glucose insulin tolerance test with minimal model analysis (FSIGTT$_{MM}$) and the euglycaemic hyperinsulinaemic clamp (EHC) technique are more precise, but also more elaborate. They are therefore not used for routine diagnostics (*Knowles* et al. 2012). For our study, it was important to elucidate if alterations in insulin sensitivity determined with an easy-to-use test could be correlated with magnesium metabolism. The OST (oral sugar test) and the OGTT (oral glucose tolerance test) (*Pratt-Phillips* et al. 2015, *Schuver* et al. 2014) also belong in this category. However, the values of these tests are influenced by gastric emptying, glucose absorption and hepatic glucose trapping (*Kronfeld* et al. 2005). Furthermore, the OST is primarily a test for β-cell response rather than a test for insulin sensitivity (*Lindåse* et al. 2017). It was found to be highly specific but had a poor sensitivity for diagnosing insulin resistance (*Dubar* et al. 2016). Some authors report a good correlation of OGTT with intravenous glucose tests, but others report strong variations in the insulin response (*Pratt-Phillips* et al. 2015, *Schuver* et al. 2014). The sensitivity of the CGIT (positive phase duration of the glucose curve > 45 minutes) was 85.7% and specificity was 40%, whereas for $I_{45} > 100\,\mu IU/mL$ sensitivity and specificity were 28.5% and 100%, respectively, when compared to the FSIGTT$_{MM}$. Hereby the CGIT showed better agreement with the FSIGTT$_{MM}$ than the OST in previous studies. Calculated parameters for the glucose curve of the CGIT should be interpreted with care, as they had low repeatability, whereas the parameters for the insulin curve had high repeatability. There was an effect of breed (Standardbred versus Icelandic horse) as well as stress on the glucose dynamics, but not on the insulin dynamics of the CGIT (*Bröjer* et al. 2013). The insulin concentrations therefore seem to be a more reliable parameter to diagnose insulin dysregulation. In our study, glucose dynamics have been used as criteria to diagnose insulin dysregulation and the influences mentioned above could have led to imprecise classifications. In the multivariable model only MIRG had a significant influence on $[Mg^{2+}]_i$. This is interesting, since proxy measures are usually recommended as screening tests, while dynamic tests are preferred for the diagnosis of insulin resistance (*Frank* et al. 2014). Nevertheless, proxies showed a better correlation with results of the FSIGTT$_{MM}$ than basal insulin concentration and HOMA-IR (*Treiber* et al. 2005). It would be of interest to examine the influence of a magnesium supplementation on parameters of ID, including MIRG.

Finally, this study was not able to identify sex differences in the magnesium status of EMS horses. In human medicine, a higher incidence of hypomagnesemia could be shown in women compared with men at a 2-to-1 ratio (*Pham* et al. 2005, *Sheehan* 1991). In addition, men with diabetes may have higher levels of ionized magnesium (*Mikhail* and *Ehsanipoor* 1999). In the horses of the present study, however, $[Mg^{2+}]_i$ was not significantly different between sexes. It remains to be shown whether the latter may be attributable to the fact that all male horses of the present study (15/38) were geldings and, as such, not under the metabolic influence of testosterone.

In conclusion, results of this study showed a lower intracellular magnesium concentration in horses with ID without sex differences. We further demonstrated a positive correlation between the intracellular magnesium concentration and the proxy MIRG. Further studies should examine the role of magnesium metabolism in horses with metabolic syndrome, especially in horses with more pronounced insulin resistance.

List of abbreviations

BCS	body condition score
BW	body weight
cAMP	cyclic adenosinmonophosphate
CGIT	combined glucose insulin tolerance test
CNS	cresty neck score
EHC	euglycaemic hyperinsulinaemic clamp
EMS	equine metabolic syndrome
FSIGTT$_{MM}$	frequently sampled glucose insulin tolerance test with minimal model analysis
G_0	baseline glucose
GGT	gamma-glutamyl transferase
HOMA Index	homeostasis model assessment for insulin resistance
I_0	baseline insulin
I_{45}	insulin after 45 minutes
I_{45-0}	insulin difference
ID	insulin dysregulation
IFG	impaired fasting glycaemia
IGT	impaired glucose tolerance
IQR	inter quartile range
$[Mg^{2+}]_i$	intracellular magnesium concentration
MIRG	modified insulin-to-glucose ratio
OGTT	oral glucose tolerance test
OST	oral sugar test
PI3K	phosphoinositide 3-kinase
PKA	protein kinase A
PKB	protein kinase B
RISQI	reciprocal inverse square of insulin
SD	standard deviation
SLC41A1	solute carrier family 41, member A1
TG	triglycerides

Conflict of interest statement

The authors declare not to be subject to any conflicts of interest in regard to drugs or technical equipment used in the present study.

Animal Welfare Statement

Statement of informed consent

Acknowledgment

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