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Evaluation of Selected Serum Biochemical Constituents and Combined Glucose Insulin Test in Equine Metabolic Syndrome Affected Ponies

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Abstract

This study was performed to compare between healthy and equine metabolic syndrome (EMS) regarding serum activity of GGT, CK, LDH, triglycerides, total protein, albumin, globulin concentrations as well as fasting insulin and fasting glucose levels.12 ponies (control, n=5 and EMS cases, n=7) were examined physically and biochemically at private sector in Giza Government. Combined glucose insulin test was performed to estimate insulin sensitivity in EMS ponies. Ponies of 7 – 9 body condition score (BCS) suffered from EMS showed significant increase in serum GGT activity ($P<0.01$), triglycerides ($P<0.01$), fasting glucose ($P<0.05$) and fasting insulin level ($P<0.05$) with no significant increase in serum CK activity and decrease in serum LDH activity. The application of CGIT showed significant increase ($P<0.001$) in serum insulin and glucose levels at the minute 45when compared with zero time which confirmed the insulin resistance. The obtained data can be used in the diagnosis of EMS cases.

Keywords: CGIT, EMS, Insulin, Ponies, Serum biochemistry.

Introduction

Equine metabolic syndrome (EMS) was first referred by Johnson in (2002). It is not a specific disease, but rather a clinical syndrome associated with laminitis (Treiber et al., 2006; Carter et al.,2009).The use of the EMS term is especially practical for distinguishing affected horses from other confusing illness like Cushing's disease or hypothyroidism, with which EMS is often confused (Johnson, 2002). Increased adiposity with hyperinsulinemia, and insulin resistance (IR), the three main components of this syndrome are not easily being separated from other previous illness (Frank, 2011). Neither all EMS-affected horses are obese nor all obese

horses develop IR (Treiber et al., 2006; Vick et al., 2006; Johnson et al., 2010).Other components of EMS include dyslipidemia, altered blood dipokine concentrations, systemic inflammation, and seasonal arterial hypertension (Kearns, 2006; Treiber et al. 2006; Vick et al. 2007; Bailey, 2008). Insulin resistance is a key feature of EMS, therefore reasoning the condition as an endocrine disorder (Frank, 2010).

Laminitis is the most important finding in EMS because of its negative effect on the animal's welfare. Subclinical laminitis takes the form of divergent growth rings (*founder lines*) on the hooves or radiographic evidence of either third phalanx rotation or sinking. This is sometimes described as *endocrinopathic laminitis* because of its association with EMS and Cushing's disease (Frank, 2010). Identification of EMS affords help to recognize horses with potential ability to develop laminitis and to make changes in management practices for insulin resistant horses that with possible reproduction problems (Earl, 2011).

Combined glucose insulin test (CGIT) is more sensitive than resting glucose and insulin measurements (Frank et al., 2006). CGIT is a qualitative test used to estimate insulin sensitivity in horses (Eiler et al., 2005). IR is diagnosed by detecting a blood glucose concentration higher than baseline at the minutes 45 or an insulin concentration greater than 694.5 pmol/L at the same time point (Frank et al., 2006). This work was carried out to follow the blood biochemical alterations and apply CGIT in ponies suffering from EMS to assess insulin sensitivity to be used as a guide for diagnosis.

Material and methods

This study was carried out on 12 ponies included five healthy ponies (control group) and seven showing EMS phenotypes (EMS group), their ages ranged between 3 - 19 years with body condition score (BCS) ranged from 6 to 9 according to scale described by Henneke et al. (1983). Animals were fed on unmeasured ration according to the condition of each farm. All ponies received routine deworming program before the start of the study. Each animal was exposed to clinical examination and blood samples were collected at least 6 hours after their last meal. Blood was collected via jugular venipuncture using a 20-G needle and two sterile Vacutainer tubes. One plain tube to obtain serum for biochemistry and one with sodium fluoride to obtain plasma for glucose concentrations. Tubes

were centrifuged at 1500 rpm for 15 minutes, then plasma or serum were collected and frozen at –20°C.Samples were analyzed for plasma glucose level, serum triglycerides, gamma glutamyl transferase, creatine phosphokinase and total protein levels using APEL spectrophotometer – PD – 303S – Japan, with the specific kits produced by Stanbio Company.

Serum insulin concentrations were determined through radio-immunoassay using ELISA reader –Dynatech Product – USA, with human insulin ELISA kit produced by Mercodia company according to the method described by Öberg et al., (2012) who stated that the equine assay correlated well with both human assays (one ELISA and one RIA).

Combined glucose insulin test (CGIT) was applied on 6 ponies (three healthy and three showing EMS phenotypes) after fasting for at least 6 hours, intravenous catheter was applied to obtain a pre-injection sample for baseline glucose and insulin measurements. Injection of 150 mg/kg b.wt dextrose in 50% solution, immediately followed by 0.10 U/kg b.wt of regular insulin (Humulin R; Eli Lilly). Blood glucose concentrations were measured at 1, 5, 15, 25, 35, 45, 60, 75, 90, 105 and 120 minutes post-infusion. A blood sample was collected at 45 minutes post injection and submitted for the second insulin (Frank, 2010).

Obtained data were analyzed using ANOVA test using SPSS, statistical program version 16.1and tabulated as mean±SEM at levels of significance $P \leq 0.001$, $P \leq 0.01$ and $P \leq 0.05$; the values have the same symbol are not significantly different.

Results

Significant increase in GGT($P<0.01$), triglycerides ($P<0.01$), fasting glucose ($P<0.05$) and fasting insulin ($P<0.01$),insignificant increase in CK and insignificant decrease in LDH in EMS ponies, while no changes in total protein, albumin and globulin levels between two groups (Table 1).

The level of glucose in both EMS and healthy ponies decreased continuously along the time of the test. At 45 minute, the glucose level was higher than that at zero time in EMS ponies and significantly increased ($P<0.001$) in comparison with healthy ponies (Table 2 and Figure 1). At 45 minute, the insulin level was higher than that at zero time in EMS ponies and significantly increased ($P<0.001$) in comparison with healthy ponies (Table 3 and Figure 2).

Table 1. Selected serum biochemical constituents, fasting glucose and insulin in EMS and healthy ponies.

Parameters	EMS (n=7)	Control (n= 5)
GGT (U/L)	27.5±1.726 [b] (19-33)	17.95±0.579 (17-19)
CK (U/L)	162.4±31.126 (91-354)	147.42±6.266 (135-155)
LDH (U/L)	150.44±11.857 (111-206)	168.24±28.352 (122-219)
Triglycerides (mmol/L)	0.769±0.108 [b] (0.384–1.322)	0.399±0.044 (0.316–0.452)
Total protein (g/L)	71.7±1.62 (70 – 80)	67. 9±2.71 (60-70)
Albumin (g/L)	37.8±1.76 (30 – 40)	34±3.12 (30 – 40)
Globulin (g/L)	33.9±1.77 (30 – 40)	33.8±3.22 (30 – 40)
Fasting glucose (mmol/L)	5.3913±0.125[c] (4.995 – 5.88)	4.86±0.210 (4.50 –5.22)
Fasting insulin (pmol/L)	113.342±14.168 [b] (69.45 –166.68)	59.102±12.008 (41.67– 83.34)

a: P<0.001; b: P<0.01; c: P<0.05

Table 2.Combined Glucose Insulin Test (glucose mmol/L) in EMS and healthy ponies.

Time of sampling (min)	EMS (n=3)	Control (n=3)
Zero time*	5.28±0.189 (5.05 – 5.661)	4.99±0.070 (4.88 –5.106)
1st	17.05±4.766 (11.71– 26.585)	11.391±0.160 (11.1–11.655)
5th	10.72±0.311 (10.10 – 11.04)	9.609±0.135 (9.379–9.8235)
15th	9.487±0.529 (8.436 – 10.156)	6.273±0.088 (6.105–6.438)
25th	8.254±0.134 (8.05 – 8.491)	5.574±0.0784 (5.439–5.7165)
35th	7.993±0.067 (7.936–8.103)	5.075±0.0714 (89–94)
45th	7.934±0.427 a (6.438 –7.881)	4.502±0.0824 (4.329–4.606)
60th	6.893±0.526 (5.994 –7.936)	4.747±0.0668 (4.606–4.884)
75th	6.317±0.322 (5.883 – 6.937)	4.130±0.06 (4.05–4.273)
90th	6.079±0.127 (5.827–6.2715)	4.105±0.0578 (3.996–4.218)
105th	5.77±0.221 (5.328–5.994)	3.821±0.053 (3.718–3.940)
120th	5.507±0.170 (5.1615–5.7165)	3.777±0.0532 (3.663–3.885)

*Zero time = after fasting 6 hours
a: P<0.001; b: P<0.01; c: P<0.05

Table 3. Combined Glucose Insulin Test (insulin pmol/L) in EMS and healthy ponies.

Time of sampling (min)	EMS (n=3)	Control (n=3)
Zero time *	158.346±3.167 (152.79 –166.68)	81.117±1.18 (69.45–83.34)
45th	1830.91±160.65 a (1534.85–2083.5)	264.74±3.715 (256.965–270.855)

*Zero time = after fasting 6 hours
a: P<0.001; b: P<0.01; c: P<0.05

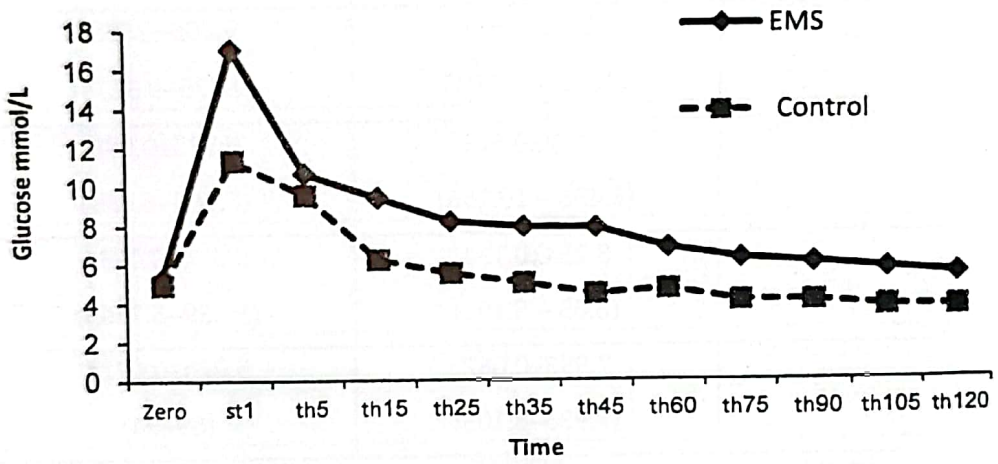

Figure 1. Combined Glucose Insulin Test (glucose mmol/L) in EMS and healthy ponies

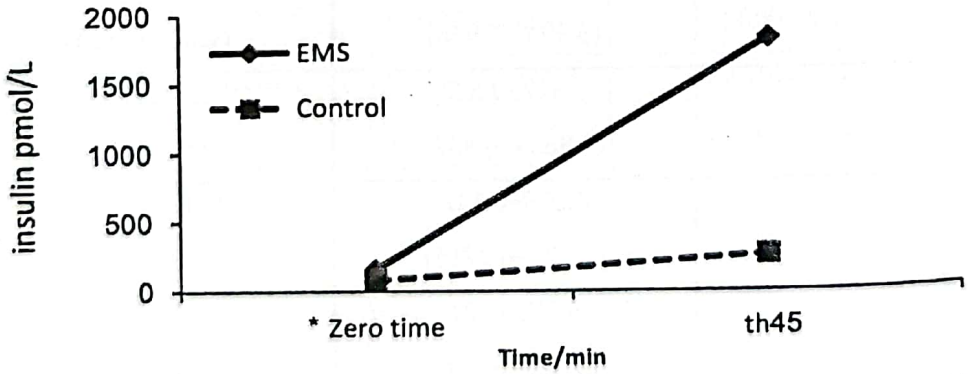

Figure 2. Combined Glucose Insulin Test (insulin pmol/L) in EMS and healthy ponies

Discussion

EMS is not a specific disease entity but rather clinical syndrome associated with insulin resistance, hyperinsulinemia and hypertriglyceridemia which prone to laminitis. In control group, the activity of GGT ranged between 17-19 U/L, that value came in accordance with Smith (2009), Barton (2010) and Robinson and Sprayberry (2009), while Kaneko et al., (2008) reported lower values. The activity of CK ranged between 135-155 U/L, similar values were recorded by Rockett and Bosted (2007) while Kaneko et al., (2008) recorded low values and Smith (2009) recorded higher values. The activity of LDH ranged between 122 – 219U/L which came in accordance with Robinson and Sprawberry (2009), while Kaneko et al., (2008) and Smith (2009) recorded higher values. The level of triglyceride ranged between0.316–0.452mmol/Land that in accordance with Kaneko et al., (2008). The level of total protein ranged between 60-70 g/Land that in accordance with Rockett and Bosted (2007); Radostits et al., (2007); Robinson and Sprawberry (2009); Smith (2009). The level of albumin and globulin ranged between 30 – 40 g/L and that in agreement with Kaneko et al. (2008) and Robinson and sprawberry (2009). The level of glucose ranged between 4.50 –5.22 mmol/L, Similar values were recorded by Kaneko et al. 2008; Smith 2009; Radostits et al., 2007. The level of insulin ranged between41.67– 83.34pmol/L, while Frank et al., (2006) ranged insulin level between 44.448–146.54 pmol/L and Frank (2010) mentioned that normal insulin level is <138.9 pmol/L.

In ponies suffering from EMS, significant increase in GGT was observed during the study which can be explained by lipid accumulation in the liver as a result of high free fatty acids (FFA) concentrations can be implicated (Wasada et al., 2008).Insignificant decrease in LDH was not clinically important(Barton, 2010).Insignificant increase in CK was estimated as usual equine diet have slight amount of fat and excess glucose can be transformed into fat by de novo lipogenesis; fats are used for energy production or stored as triglyceride inside cells. When the storage capacity of adipose tissues is exceed, fats are repartitioning in skeletal muscle, liver, and pancreatic tissues resulting in lipid accumulation within these tissues and alter normal cellular functions and that referred to *lipotoxicity* (Slawik *and* Vidal-Puig, *2006).*

Significant increase in triglyceride level was observed; normally, insulin inhibits fat mobilization by inhibiting the activity of hormone-sensitive

lipase and promotes the uptake of triglycerides into peripheral tissues by stimulating the activity of lipoprotein lipase. Overproduction of triglycerides was assumed to be a result of defective catabolism although the exact mechanism is unknown (Waitt and Cebra, 2009).

Significant increase in fasting insulin level was observed which can be referred to that insulin secretion from the pancreas increases to compensate for reduced tissue sensitivity and euglycemia is maintained. However, recent results from the author's laboratory show that resting hyperinsulinemia can't be attributed to increased insulin secretion in all cases, which suggests that insulin clearance may be affected in some animals (Frank, 2010).

Significant increase in fasting glucose level was observed and that indicate blood glucose levels increased as IR progressed (Frank et al., 2006).

In CGIT, the level of glucose at zero time was ranged between 5.05 – 5.661 mmol/L with mean value of 5.28±0.189 in EMS group, while the control was ranged between 4.884 –5.106mmol/L with mean value of 4.99±0.070.There was sharp increase in glucose level reach to 17.05±4.766 in EMS group compared to control group (11.391±0.160) at the 1st minute post injection of 150 mg/kg 50% dextrose, immediately followed by 0.10 U/kg b.wt of regular insulin intravenous, a continuous decrease in glucose level was obvious until 120thminute post injection. At 45th minute the blood glucose concentration was higher than zero time in EMS group, while in control group blood glucose concentration was lower than zero time, which is indicative for the insulin resistance and the failure of insulin to induce the expected responses in target tissues by stimulate glucose uptake (Frank et al., 2006).In this experiment, ponies classified as IR not only by failure to return to pre-treatment concentration (zero time) by 45 min, but also failure to return to pre-treatment concentration by the end of the 120-min post test(Caltabilota2009). The level of insulin at zero time was ranged between 152.79 –166.68 pmol/L with mean value of 158.346±3.167, while the control was ranged between 69.45–83.34 pmol/L with mean value of 81.117±1.18, at 45th minute there was sharp increase in insulin level reached1830.91± 160.65 in EMS group compared to control group 264.74±3.715, insulin level higher than 694.5 pmol/L was considered the hallmark of insulin resistance diagnosis (Frank et al., 2006).

Conclusion

Regarding to the serum biochemical constituents ponies of 7 – 9 BCS, suffered from EMS had significant increase in GGT ($P<0.01$), triglycerides ($P<0.01$), fasting glucose ($P<0.05$) and fasting insulin ($P<0.05$) and increase in CK and decrease in LDH. The application of CGIT showed significant increase ($P<0.001$) in insulin and glucose at 45 minute than zero time, which confirmed the insulin resistance. Obtained data can be used in the diagnosis of EMS cases.

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تقيم مكونات بيوكيميائيه منتقاه و اختبار الجلوكوز و الانسولين المصاحب فى البونى المصاب بالمتلازمه الايضيه الخيليه

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أجريت هذه الدراسة للمقارنة بين البونى السليم والمصاب بالمتلازمة الايضية الخيلية بالرجوع إلى نشاط انزيمات الجاما جلوتاميل ترانسفيريز والكرياتينين كاينيز وكذلك مستوى الجليسريدات الثلاثية والبروتين الكلى والزولال والبروتين فى مصل الدم بالاضافة إلى مستوى الانسولين الصائم والجلوكوز الصائم. تم فحص 12 بونى (مجموعة ضابطة عدد 5 ومجموعة مصابى عدد 7) فحصًا فيزيائيًا و بيوكيميائيًا فى اسطبلات خاصة بمحافظة الجيزة. اختبار الجلوكوز والانسولين المصاحب أجري لاستبيان حساسية الانسولين فى البونى المصابة.

أظهرت البونى ذات تقييم حاله الجسم بين 7 الى 9 المصابة وجود زيادة معنوية فى نشاط انزيم الجاما جلوتاميل ترانسفيريز و مستوى الجليسريدات الثلاثيه ($p<0.01$) ومستوى الجلوكوز الصائم والأنسولين الصائم ($p<0.05$) مع زيادة فى نشاط انزيم الكرياتينين كاينيز ونقص فى نشاط انزيم لاكتيت هايدروجينيز. إن تطبيق اختبار الجلوكوز والأنسولين المصاحب أظهر زيادى معنوية فى مستوى انسولين و جلوكوز مصل الدم ($p<0.001$) عند الدقيقة 45 وذلك بالمقارنة مع المستوى الصائم وهذا يؤكد حدوث مقاومة الأنسولين.

هذه النتائج المتحصل عليها يمكن استخدامها فى تشخيص حالات المتلازمة الايضية الخيلية.