Acta Scientific Veterinary Sciences (ISSN: 2582-3183)

Volume 2 Issue 3 March 2020

Research Article

Assessment of Insulin Resistance Diagnostic Techniques in Equines in the City of Mineiros – GO

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DOI: 10.31080/ASVS.2020.02.0045

Received: January 29, 2020

Published: February 06, 2020

Abstract

Insulin sensitivity is a determinant factor for the development of equine metabolic syndrome. The identification of more accessible methods to be used in clinical practice is essential to enable early intervention of animals with this condition. The objective of this experiment was to measure insulin values, basal glucose, proxies and analyze their behavior in the equine population located in the city of Mineiros-GO. A total of 74 horses were evaluated to analyze glycemia, insulinemia and the HOMA-IR, RISQI, MIRG and G: I proxies in all animals separated by age group, body condition and by gender. For the statistical analysis the data with normal distribution were analyzed by analysis of variance, followed by the Tukey test. The results were submitted to analysis of variance, with Tukey-Kramer post-test, the analysis of the coefficient of correction of proxies, ($P \leq 0.05$). It was observed that the insulin sensitivity in the equine population of this study is lower when compared to other studies, with alterations in blood glucose values between foals with other age groups ($p<0.05$) and alteration between proxies G:I, MIRG and RISQI among the lean to optimal body condition group ($p<0.05$).

Keywords: Horses; Obesity; Metabolic Syndrome

Introduction

When the subject insulin resistance (IR) is reported, it must be remembered that this condition is related with other alternations, such as glucose tolerance, plasmatic dyslipidemia and hypertension, all this phenotypical alternations are a part of metabolic syndrome [1]. But, what actually is insulin resistance? To Shanik and collaborators [2] this term must be used when the cell surface is insensitive due to dependent insulin tissues intracellular changes, mainly in musculoskeletal, adipose and hepatic tissues and it's intimately related to hyperinsulinemia.

Insulin resistance it's not uncommon in equines, in a experiment performed in the USA it was noted in 10% of the horses and a randomly chosen population was noticed hyperinsulinemia, in another study, performed in Australia, the number of equines with hyperinsulinemia reached 28% [3]. It may be a physiological response to situations like pregnancy and stress moments, insulin also suffers elevation in response to insulin post receiver's counter regulation actions to progesterone, estradiol, growth hormone, placental lactogen and placental cytokines [4].

In equines insulin resistance is related to obesity and have been correlated to endocrinopathy laminitis' physiopathology and to glucose metabolism [5].

Insulin deregulation diagnosis and it's insensitive can be performed through tests that consider both insulin and glucose, there are 2 types of tests, the static ones, which are based on glucose and insulin basal levels and dynamic tests, which are based on the organism's response to glucose and/or insulin. The dynamic ones are more complex and involve serial blood collection, making this exams more expensive and also needing more time to it's execution, however they are more trustworthy [6]. The use of alternative means that make the insulin resistance diagnosis easier it's necessary to veterinarians that work in the field [7].

An alternative to these dynamic tests it's indirect indicators or ''proxies'', that are obtained trough formulas that use insulin and glucose basal values to determinate if animals present situations of sensibility or insulin resistance [8].

This experiment aims to measure insulin and glucose basal values, and calculate the ‘’proxies’’ HOMA-IR, RISQI, MIRG, G:Iand to analyze it’s behavior in equines population located in the city of Mineiros- GO.

Materials and Methods

This project was approved by the Ethics Committee in Animals Use (CEUA) in the University Center of Mineiros, with protocol number 18-2017. Equines located in the city of Mineiros in the state of Goías (17º 34' 10" S e 52º 33' 04" W) were analyzed, with venous blood collect of 74 animals, being 47 females and 27 males, with age average of(±DP) de 5.91 (±3.84).The evaluated animals were distributed in the following races: Quarto de milha, Mixed Breed (SRD), Piquira e Pampa.

Animals height (P) was obtained with weighing tape, having as a measure thoracic circumference compared with metric tape and positioned right after the end of the withers, between the spinal process T8 and T9 vertebrae, going trough intercostal space of ribs, until last rib articulation with xiphoid process. To measure body condition score it was used a scale that goes from 1 to 9 (1= emaciated animal and 9= extremely obese animal) according to graduation proposed by Henneke., *et al*. [9].

The collect was made by external jugular vein puncture in vacuum collection tubes (vacutainer®) without 10 ml anticoagulant (to serum analysis)and with 3 ml fluoride (to plasma analysis). After collected, the tubes were frozen to -20°C and sent to analysis in the clinical laboratory located in Experimental Farm Luis Eduardo de Oliveira Sales - FELEOS which belongs to University Center of Mineiros - UNIFIMES.

The tests were done by spectrophotometric identification system in automated biochemical analyzer (COBAS MIRAGE-ROCHE DIAGNOSTIC SYSTEM®) using specific commercial Kits (Bioclin®), blood samples packed in bottles without anticoagulant were used to get silky insulin profile, 1000 g being centrifuged, during 10 minutes, to serum separation, to insulin analysis, Cobas commercial Kit was used by electrochemiluminescence method (immunoassay or “ECLIA”) (ref. 12017547 122).From each blood sample picked with fluoride, plasma was used to measure plasmatic glucose values (colorimetric method – enzymatic – ref. K082).

To insulin measures in fasting were considered as reference value until 20 μUI/Ml. To plasmatic glucose in fasting was considered as reference value the interval between 70 a 135 mg/dL [10].

Proxies used in this experiment were based in real glucose and insulin basal values, being the relation among glucose and insulin (G.I.), the sensibility to insulin (RISQI), the insulin secretory response by pancreatic β cells (MIRGI) and the homeostasis evaluation model (HOMA-IR) following Treiber., *et al.* [8] methodology, with it’s respective formulas described bellow:

G:I = glucose/insulin

RISQI = 1/√insulin = insulin-0.5

MIRG = (800 – 0.3 [insulin – 50]2)/(glucose – 30)]

HOMA – IR = glucose (mg/dL) x 0.0555 x insulin (μU/ml)/22.5

As normality parameters to proxies, it was considered relation value of G:I > 10 normal, RI compensatory between 4.5 and 10 and severe picture of RI if < 4.5 [8]. To RISQI normal > 0.32, between 0.22 and 0.32 RI and < 0.22 RI severe. To normal MIRG < 5.6 and to HOMA-IR, it was considered RI when value was superior to 2.71 [11].

To data analysis, animals were grouped according to it’s gender, with 27 males and 47 females, about age group, divided in three groups, group 1, equines considered foal with age between 1 and 3 years old with average age of 2.2 (±0.64), in group 2 we can find horses considered young, with age between 3 and 6 years, with average age of 5.01(±0.86) and at last group 3, denominated adult horses, with age between 7 and 18 years old, with average age of 10.54 (±2.93)and by it’s score in body condition, being categorized in thin (ECC < 4) with 23 animals, great body condition (CCO) (ECC 4.5 – 6) with 42 horses, over weight (ECC 6.5 – 7) with 3 animals and obese (ECC 7.5 – 9) with a total of 6 horses [12].

To statistical analysis, quantitative variables were presented as average and standard deviations. The determination of differences among experimental groups were performed through Variance Analysis (ANOVA) two-tailed test, with Tukey-Kramer post-test, was performed pearson’s correlation coefficient analysis among proxies and Turkey’s test among variables, with assistance of SAS University program, version 3.71, considering significant the differences with p≤0.05.

Results

A total of 74 animals were evaluated, distributed in 47 (63.51%) females and 27 (36.49%) males, animal’s division and it’s respective percentages among age groups were: foal (age 1 – 3 years old) 24 animals (32.43%), young animals (age 3 – 6 years old) 26 animals (35.14%) and adults individuals (age 7 – 18 years old) with a total of 24 equines (32.43%), the body condition group’s distribution are visible in table 1. None of the evaluated animals in this experiment presented clinic alternation in it’s physiological parameters.

Body Condition	Total	
	N	%
Thin	23	31.08
Great	42	56.76
Overweight	3	4.05
Obeses	6	8.11

Table 1: Number of animals relation and it's respective values corresponding to body condition percentage of the 74 equines located in city of Mineiros - GO.

Thin Animals (ECC<4), Great body condition (ECC 4.5 – 6), Overweight (ECC 6.5 – 7), Obsess (ECC 7.5 – 9).

When it came to insulin variable, just one animal presented hyperinsulinemia case, representing 1.36% of all animals, being a mare, young with 6 years old and with a ECC of 6, demonstrating alternations in all measured proxies measured in this experiment.

The insulin and glucose basal values together with calculated proxies in this measured experiment in all animals are described in table 2.

	N	Average	DP	Inferior Limit (IC=95%)	Upper Limit (IC=95%)
Glucose (mg/dL)	74	85.05	16.18	81.3	88.8
Insulin (µU/mL)	74	3.66	4.57	2.6	4.72
HOMA-IR	74	0.86	1.2	0.58	1.14
G:I (mg/dL/µU/mL)	74	70.43	81.35	51.58	89.28
RISQI (µU/mL-0,5)	74	0.82	0.5	0.71	0.94
MIRG (µUins2/[10.L.mggli])	74	2.62	1.46	2.28	2.96

Table 2: Proxies' values, basal glucose and insulin of 74 females and males equines located in the city of Mineiros-GO.

G:I: Relation Between Glucose and Insulin; RISQI: Sensibility to Insulin; MIRG: Secretory Insulin Response; HOMA-IR: Homeostasis avaluation Model.

Average values and it's respective standard deviation of gender comparison among basal glucose and insulin variables and proxies did not present significant statistics alternations, as showed in table 3.

	N	Glucose (mg/dL)		Insulin (µU/mL)		HOMA-IR		G:I (mg/dL/ µU/mL)		RISQI (µU/mL-0,5)		MIRG (µUins2/[10.L.mggli])	
		Average	DP	Average	DP	Averge	DP	Average	DP	Average	DP	Average	DP
Females	27	85.78	17.12	4.02	5.37	0.95	1.39	75.39	86.65	0.84	0.53	2.72	1.73
Inferior Limit (IC=95%)		80.75		2.44		0.54		49.94		0.69		2.21	
Upper Limit (IC=95%)		90.81		5.60		1.36		100.83		1.006		3.23	
Males	47	83.77	14.60	3.03	2.64	0.7	0.76	61.79	71.94	0.79	0.44	2.44	0.79
Inferior Limit (IC=95%)		77.99		77.99		0.39		33.34		0.61		2.13	
Upper Limit (IC=95%)		89.55		89.55		1.006		90.25		0.96		2.76	
P Value		0.61		0.37		0.38		0.49		0.65		0.43	

Table 3: Average values comparison and proxies' standard deviation (DP), basal glucose and insulin among 27 mares and 47 males equines located in the city of Mineiros-GO.

Turkey's Test, significant P value ≤0,05. Thin Animals (ECC<4), Great body condition (ECC 4.5 – 6), overweight (ECC 6.5 – 7), obese (ECC 7.5 – 9). G:I: Glucose and Insulin Relation; RISQI: sensibility to Insulin; MIRG: Secretory Insulin Response; HOMA-IR: Homeostasis Avaluation Model.

When variables are analysed according to age groups it is possible to note the significant difference in plasmatic glucose values, in comparation among foals, young and adults age group (p = 0,01) as showed in figure 1, other parameters did not present significant difference (Table 4).

Animals with great weight presented significant difference compared to animals considered thin in relation to glucose and proxies G:I, RISQI e MIRG (Table 5).

After checking proxies' parameters classification reference, it was noticed a similar behavior among them, related to groups between gender and age, not demonstrating statistic difference. After analysing groups by body condition, it was possible to notice statistic difference between thin group and great body condition among proxies: G:I, RISQI and MIRG with value p<0,05. All proxies are correlated with each other, with a r high value and a p significant value, as showed in table 6.

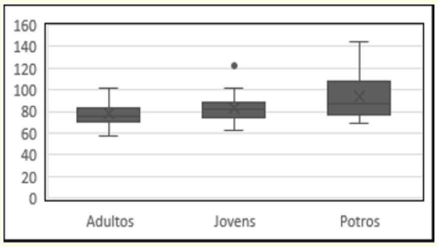

Figure 1: Average and plasmatic glucose values (mg/dL) standard deviations (DP) related to 74 equines age groups located in the city of Mineiros-GO.

Foals (age 1 – 3 years old), Young (age 3 – 6 years old), Adults (Age 7 – 18 years old). Different letters represent significant statistics differences among groups, with p ≤ 0.05.

Age	Foals		Young		Adults	
	Average	DP	Average	DP	Average	DP
Glucose (mg/dL)	93.58[a]	20.3	83.65[b]	12.58	78.04[b]	10.82
Insulin (µU/mL)	3.99	3.78	4.39	6.37	2.54	2.44
HOMA-IR	1.04	1.2	1.004	1.56	0.52	0.54
G:I (mg/dL/ µU/mL)	64.25	78.48	67.3	0.8	80.00	87.3
RISQI (µU/mL-0,5)	0.75	0.48	0.8	0.49	0.92	0.53
MIRG (µUins2/[10.L.mggli])	2.46	1.14	2.9	1.95	2.48	1.07

Table 4: Proxies' values average and standard deviations (DP), glucose and insulin, related to 74 equines age groups located in city of Mineiros-GO.

Foals (Age 1 – 3 years old), Young (Age 3 – 6 years old), Adults (Age 7 to 18 years old). G:I: Glucose and Insulin Relation; RISQI: Sensibility to Insulin; MIRG: Secretory Insulin Response; HOMA-IR: Homeostasis Avaliation Model. Different letters represent significant statistics differences among groups, with p ≤ 0.05.

Discussion

Equines used in this study can be characterized as heterogenous group, coming from different properties and having different diets, with 87.84% of the animals being considered thin and in great body condition and only 12.16% considered overweight and obese.

Body Condition	Thin		Great		Overweight		Obese	
	Average	DP	Average	DP	Average	DP	Average	DP
N	23		42		3		6	
Glucose (mg/dL)	78.73[a]	15.06	89.85[b]	17.28	80.33[b]	6.5	78[b]	5.4
Insulin (µU/mL)	1.88	1.88	4.82	5.38	2.74	1.85	2.8	1.85
HOMA-IR	0.44	0.44	1.15	1.41	0.56	0.41	0.54	0.36
G:I (mg/dL/µU/mL)	132.05[a]	110.69	41.05[b]	39.53	40.93	28.16	54.63	63.54
RISQI (µU/mL-0,5)	1.22[a]	0.61	0.63[b]	0.31	0.69	0.27	0.75	0.42
MIRG (µUins2/[10.L.mggli])	1.97[a]	0.82	2.97[b]	1.7	2.51	0.7	2.7	1.01

Table 5: Proxies' value average and standard deviations, glucose and insulin, related to 74 equines' body condition located in the city of Mineiros – GO.

Thin Animals (ECC<4), Great body condition (ECC 4.5 – 6), Overweight (ECC 6.5 – 7), Obese (ECC 7.5 – 9).

Subtitle: a. Significant difference between thin group and great group. G.I: Relation Between Glucose and Insulin; RISQI: Sensibility to Insulin; MIRG: Secretory Insulin Response; HOMA-IR: Homeostasis Avaliation Model. Different letters represent significant statistics differences among groups, with p ≤ 0.05.

	HOMA-IR		G:I (mg/dL/ µU/mL)		RISQI (µU/mL-0,5)		MIRG (µUins2/[10.L.mggli])	
	r	p	r	P	r	p	R	p
HOMA-IR	-	-	0.826	<0.001	0.79	<0.001	0.65	<0.001
G:I (mg/dL/ µU/mL)	0.826	<0.001	-	-	0.86	<0.001	0.82	<0.001
RISQI (µU/mL-0,5)	0.79	<0.001	0.86	<0.001	-	-	0.79	<0.001
MIRG (µUins2/[10.L.mggli])	0.65	<0.001	0.82	<0.001	0.79	<0.001	-	-

Table 6: Correlation indexes among proxies' variables from 74 male and female equines located in Mineiros-GO.

Person's test (P ≤0.05). G:I: Glucose and Insulin Relation; RISQI: Sensibility to Insulin; MIRG: Secretory Insulin Response; HOMA-IR: Homeostasis Avaliaton Model.

Literature has demonstrated that there is a huge variation among insulinemia and glycemia results, under diet, gender, race, type of management, athletic activities and region where they were raised influences, besides technical laboratory differences used to this variables evaluation, which makes necessary some precaution when comparing academic works [13].

Insulin values do not statistically differ among gender, age and body condition groups, agreeing with Nadeau's results and contributors [14] and Turner [15], in contrast Mello [16] observed significant difference among groups with fat deposition, age and rearing system.

Equine's percentage with hiperinsulinemia in animals used in this research was 1.36% from total of animals, inferior value than observed by authors like McGowan e McGowan [3] that observed between 10 and 28% in analyzed population respectively, this contrast can be justified by heterogeneous characteristics from animals used in this experiment and by the fact that insulin it's not a specific indicator to sensibility in equines [17].

Insulin average values from equine's population in Mineiros are above than observed by Ramalho and Ramalho and collaborators [18] that was 1.95 (DP:1.47) μU/mL, where it was analyzed a population composed by only 10 males from the same property, these values also were above from results obtained when compared to males in this experiment. However basal insulin values in this experiment are in the same interval observed by Nadeau., *et al.* [14], Ralston [19], Dugat., *et al.* [20].

After evaluating all the animals glucose values in these experiment it was clear that interval among values match studies performed by authors Moreira., *et al.* [21], Nadeau., *et al.* [14] and Turner [15]. It wasn't noticed statistic difference in glycemia values in genders, agreeing with data provided by Turner [15] and disagreeing with (2016) that noticed values alternations in basal glycemia among males and females.

On the other hand, after analyzing basal glycemia among animals in different age groups, it was possible to notice that animals considered foals, presented average value superior to young and adults, agreeing with data obtained by Mello [16] and Murphy., *et al.* [22], this behavior can be justified due to metabolic organism rate, this metabolic rate in equines it's determined by growth hormone (GH), it is related to carbohydrate metabolism exercising antagonistic action to insulin, having as consequence a increase in glycemia, and GH presents higher concentration in foals [23,24].

Animals with thin body condition presented plasmatic glucose average values inferior to other three groups, statistically considered significant ($P<0.05$), agreeing with work from Mello [16], this behavior can explained by the fact that animals that posses some type of accumulation of fat present a higher insulin production by pancreatic beta cells, as higher glucose depuration consequence, due to a diet with high energy content for example, or even by a sensibility to insulin increase [25].

Reference values signalized by RISQI e MIRG in this experiment are inside superior interval noticed by Mello [16], Nadeau., *et al.* [14], Turner [15]. Treiber., *et al.* [8]. In the experiment developed by de Pratt., *et al.* [26], where animals proxies RISQI, MIRGe G: I were evaluated with corporal score between 5 and 8, it was possible to verify that values were higher when compared to obtained data in this experiment, demonstrating insulin resistance indexes, this behavior can be elucidated by the heterogenous characteristic from the population used in this study, mainly by corporal score profile.

Proxies' values in this experiment did not present statistic alternations between gender and age interval, on the other hand proxies G:I, RISQI and MIRG demonstrated significant value among thin animals in relation to animals with great body condition as observed by Mello [16].

Demonstrating that fat accumulation is a crucial factor in insulin resistance, as demonstrated by Ribeiro., *et al.* [27,28] after observing that high correlation indexes in animals with induced weight gain with MIRG, RISQI, G:I e HOMA-IR proxies alternations, but normalize during the period, signaling that animals started to demonstrate compensatory insulin resistance, which can also explain the significant non difference observed in this study in animals with obese and overweight body condition compared to other groups.

All proxies behaved similarly in this experiment, presenting high correlation indexes with r and $p < 0.0001$ value, agreeing with presented studies by Ribeiros and collaborators from 2007 and 2008, which can mean that proxies can be used as biomarkers to insulin sensibility.

Proxies are practical tools to insulin resistance diagnosis and according to Morgan., *et al.* [29], most used proxies in equines are RISQI and MIRG, but it is necessary to observe supported calculus in basal values, being subject to limitations in weight gain animals and suffer interference in glucose plasmatic and insulin value, but since they convert nonlinear values to linear values it is more recommended use it to monitoring animals for long periods [30].

Conclusion

Biomarkers parameters to insulin resistance in equines indicate animals metabolic condition, reference values knowledge and it's behavior to determined equine's population help in diagnosis, prognosis determination and therapeutic conduct. Results obtained in this study demonstrate that sensibility to insulin casuistry in a randomly selected population in the city of Mineiros – GO it's inferior when compared to others studies performed in other regions, but glycemia, insulinemia and proxies reference values results are similar to other studies. Glycemia behaves different regarding to age group, proxies used in this experiment demonstrate different performance among animals with different body condition and correlate with each other demonstrating the possibility of using it as viable diagnosis method to sensibility to insulin in animals with fat accumulation. The results in this study demonstrate that it is still necessary more studies about insulinic matebolism in equines.

Acknowledgement

To University Center of Mineiros (UNIFIMES) and to ANALISE laboratory for financial support.

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