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STUDY OF INTEGRAL INDICES FOR DIAGNOSING INSULIN RESISTANCE IN HORSES

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Abstract. *Since insulin resistance is a component of the pathogenesis of many diseases, including the development of metabolic syndrome, its detection and elimination should be considered as an important and necessary means of preventing diseases and their complications. Unlike many complex methods, the data of integral indices are the main ones for detecting insulin resistance disorders. Thus, the aim of the work was to diagnose insulin resistance in horses using homeostatic indices and to establish their practical significance. For the study, 18 horses were used, which were divided into 2 groups according to the scoring of physical condition, which was carried out by two independent veterinary specialists. The calculation of integral indices: HOMA-IR index, HOMA-β index, Caro index, metabolic index, atherogenicity index was performed according to the literature. It was found that the HOMA-β index and the HOMA-IR index were significantly increased by 18.1% ($p<0.01$) and 3.7 times ($p<0.01$) compared to the control; Caro index in horses with insulin resistance was reduced by 39.4% ($p<0.05$); metabolic index increased by 82.5% ($p<0.01$); atherogenicity index in horses with insulin resistance was 54.9% ($p<0.05$) higher than in control. However, when studying the ALT/AST ratio and the Matsuda index, no correlation was found. Thus, in order to assess the development of insulin resistance, appropriate indices have been proposed, the calculation of which is based on data on carbohydrate and lipid metabolism and which complement the data on the pathogenesis of this condition. However, in horses, the choice of a method for studying insulin resistance requires a thorough study.*

Keywords: insulin resistance, integral indices, horses, metabolic syndrome, obesity.

Introduction. In modern medicine and veterinary medicine, the metabolic syndrome is one of the most widely discussed problems. This syndrome is a major problem in cardiology, endocrinology, and therapy (Girvalaki, 2014). Comparative analysis of the pathophysiological mechanisms of metabolic syndrome in humans and animals indicates the presence of common pathogenesis, in particular, disorders of insulin sensitivity, glucose homeostasis and lipid metabolism. In animals, as in humans, lifestyle factors play a key role in the development of insulin resistance: physical inactivity, excessive energy intake, and chronic stress. Establishing these parallels allows the use of veterinary models to study human metabolic disorders and substantiates the prospects of translational approaches to assessing risk factors and pathogenic targets (Alemu, 2021; Bamford, 2019).

Animal models, in particular horses, dogs and rodents, have significant potential in the study of insulin resistance, given the similarity of clinical manifestations and metabolic reactions to similar processes in humans. For example, the use of the oral glucose tolerance test to assess insulin sensitivity in horses allows non-invasive monitoring of glucose metabolism, which can be adapted for screening programs in high-risk human populations. Such an interdisciplinary approach enhances the scientific understanding of the mechanisms of metabolic disorders and contributes to the improvement of preventive and therapeutic strategies in human medicine (Durham, 2021; Hukkanen, 2022).

Metabolic syndrome is a pathogenetically interrelated metabolic disorder in the body. A large complex of factors involved in the onset of this syndrome hinders accurate scientific understanding of its pathophysiological mechanisms. It was first described as an independent symptom complex of clinical manifestations in 1980 (da Silva, 2020). In 1985, S. Eaton and M. Konnor hypothesized that the increase in the number of diseases of civilization in the second half of the twentieth century was due to the fact that the genes of modern humans, adapted by a long evolutionary process to the nutritional behavior of ancestors, are not able to ensure health in the face of a dramatic change in lifestyle over the past 100 years. The intensity of stress against the background of an unbalanced diet has significantly reduced the ability to adequately adapt, not only in humans but also in animals, and formed the basis for many diseases (Daradics, 2021). Insulin resistance (IR) is closely related to the metabolic syndrome, which includes dyslipidemia, hypertension, and obesity, leading to an increased risk of cardiovascular disease. Originally proposed to describe patients with diabetes who require high doses of insulin (Er, 2016), is now defined as reduced sensitivity (maximum insulin effect) or sensitivity (insulin concentration required for a half-maximal response) to the metabolic actions of insulin, such as insulin-mediated glucose utilization by muscle and adipose tissue and inhibition of gluconeogenesis in the liver (Muniyappa, 2008). Insulin, a peptide hormone consisting of 51 amino acids, is the most important hormone for energy metabolism, such as glucose, fat, and protein, as well as for maintaining homeostasis. The most difficult to perform is the clamp test, also called the gold standard (DeFronzo, 2004). Insulin resistance is determined in different ways (Borai, 2011). The simplest ones include simultaneous determination of fasting insulin and glucose levels, which allows to assess the effectiveness of the body's tissue response to insulin, which is a key component in assessing insulin dysregulation, which is characteristic of the pathological state of insulin resistance (Park, 2021). Unlike many complex methods, the main ones for detecting insulin resistance disorders are the data of the HOMA-IR, HOMA-β index, glucose-to-insulin and glucose-to-C-peptide ratios (Goswami, 2010), as well as elevated levels of alanine aminotransferase and aspartate aminotransferase, which indicate the presence of IR (Mather, 2001). Since IR is a component of the pathogenesis of many diseases (type 2 diabetes mellitus, atherosclerosis, congestive heart failure, laminitis, etc.), its detection and elimination should be considered an important and necessary means of preventing diseases and the development of their complications (Gomez-Serrano, 2016). In our previous studies, an oral glucose tolerance test was chosen to assess insulin and non-insulin glucose dynamics because of its practicality and physiological relevance. The test assesses hyperinsulinemia and insulin dysregulation after glucose ingestion, and it has been actively used to determine insulin sensitivity in horses and ponies (Borovkov, 2024). However, modern diagnostics includes the evaluation of HOMA-IR, HOMA-β, de Ritis, metabolic index, and atherogenicity data due to their practicality and physiological significance. Thus, the metabolic syndrome as a consequence of insulin resistance is a complex problem, the solution of which should be aimed at reducing the prevalence of obesity, early detection to modify risk factors.

The aim of study. To diagnose insulin resistance in horses using homeostatic indices and to establish their practical significance.

Materials and methods. Horses of different breeds, sexes and physical condition were used for the study. Feeding and housing conditions met the physiological needs of the animals. The animals' diet was balanced in terms of essential nutrients, and all animals had free access to water and enjoyed walking. A total of 18 horses were used in the study and were divided into 2 groups based on a scoring assessment of physical condition, which was conducted by two independent veterinary specialists. All horses were kept on pasture with free access to grass hay without concentrate feeding. The horses were placed in a stall the evening before testing and given free choice of hay, grass, and water overnight; the next morning, 2 hours before testing, access to feed was restricted and a halter was placed on the horse. The calculation of integral indices, which are relevant in the study of insulin resistance, was performed according to the literature (Melnyk, 2024; Gutch, 2015).

All animal studies were conducted in accordance with the basic principles of bioethics, in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (1986) and the General Ethical Principles for Animal Experiments adopted by the First National Congress on Bioethics (2012).

The obtained results were processed by methods of variation statistics using the analysis of variance (ANOVA) software package StatPlus 5.9.8.5 (AnalystSoft Inc., USA). The reliability of the results was assessed by the Tukey's test (HSD difference of means) at the level of probability $p<0.05$, $p<0.01$ and $p<0.001$.

Results. During the studies described in detail in the previous work (Borovkov et al., 2024), all horses were divided into 2 groups according to their physical condition using a scoring system. The first group included 9 horses with an average BCS score of 4.75 (4.5-6.5), while the experimental group was formed of animals with a BCS score of 6.5 (5.5-8.0). There was no significant difference between the groups. The average age in the first group was 10.3±2.3 (8-15) years, and in the experimental group 13.5±2.6 (10-18) years. The assessment is carried out using points from 1 to 9, where 1 is a very thin horse and 9 is morphologically overweight. During the evaluation, researchers pay attention to several key areas: in healthy horses, the outline of the spine is clearly visible, while in thin horses it is more noticeable, and in obese horses, fat is felt. In good physical condition, the ribs should not be visible, but you can feel them with your fingers. In thin horses, the ribs protrude, and in overweight horses, they can be completely invisible. Hair coat and coat quality are also an indicator of physical condition: a healthy horse has a shiny, supple coat, while a thin horse has dull and tousled hair. It is also important to assess the presence and intensity of fat deposits on the sides, neck and behind the ears (Borovkov, 2024).

In the present study, the HOMA model and a set of interrelated risk factors for cardiovascular disease and insulin resistance were used to calculate the indices, which are presented in Table 1. The animal serum parameters necessary for the calculation of the relevant indices were obtained in advance and described in our previous work (Borovkov, 2024)

Table 1

Integral blood serum indices in horses with insulin resistance (n=10)

Animal groups	HOMA-IR index	HOMA-β index	Caro index	Metabolic index	Atherogenicity index
horses with IR	11,2±0,34**	75,5±0,52**	0,20±0,01*	2,30±0,03**	0,79±0,02*
control	3,0±0,15	89,2±0,48	0,33±0,02	1,26±0,01	0,51±0,03

Notes: * - $p<0.05$, ** - $p<0.01$, compared to the control

To calculate the HOMA index, the ratio of basal insulin and blood glucose concentrations is used, which reflects the balance between endogenous glucose production in the liver and insulin secretion by β-cells, which is maintained by the principle of negative feedback. This homeostatic model has two pillars: the first is the degree to which basal glucose concentration increases in response to insulin deficiency, which reflects the shape of the normal insulin secretory response to glucose; the second is that basal insulin levels are directly proportional to insulin resistance (Lim, 2019; Park, 2021). When assessing β-cell function and peripheral tissue IR using the HOMA model, significant differences between the groups were obtained. The HOMA-β and HOMA-IR indices were significantly increased by 18.1% ($p<0.01$) and 3.7 times ($p<0.01$) compared to the control. It is likely that high insulin requirements in insulin-resistant animals lead them to the manifestation of the disease and a decrease in the functional activity of β-cells (Thunander, 2011). According to the literature, in case of insulin resistance, the endocrine part of the pancreas reacts with compensatory changes in the function and mass of β-cells, which leads to hyperinsulinemia and excessive insulin production to maintain normal glucose levels (Movahedi, 2016).

Other calculated indices have been proposed to determine insulin resistance. The most famous is the F index. Caro is the ratio of fasting blood glucose to fasting insulin concentration (Caro, 1991). Thus, as a result of our study, it was found that the Caro index in horses with insulin resistance was reduced by 39.4% ($p<0.05$) compared to healthy animals. It should also be noted the well-known Bennett's index, which is calculated as 1 divided by fasting insulin and multiplied by fasting glucose (Anderson, 1995), but in horses this index is less informative. The Matsuda index should also be taken into account, which allows you to calculate insulin sensitivity. The index is calculated on the basis of fasting serum glucose (mg/dl) and insulin (mIU/l) concentrations. However, the results of the study indicate that in female animals with a history of metabolic disorders, this index is less sensitive to detect early changes associated with insulin resistance (Melnyk, 2024). This may indicate a complex mechanism that regulates glucose homeostasis and correlates with hormonal variations, which requires further investigation (Lechner, 2021).

The state of insulin resistance, in turn, causes dysregulation of lipid metabolism, which is manifested not only in changes in the lipid spectrum of the blood (which is usually determined in patients), but also in changes in the fatty acid spectrum of circulating lipid fractions (Lonardo, 2005). According to the current literature, it is known that the development of cardiovascular disease is a consequence of insulin resistance, which affects the depth of the processes. Since IR is of high clinical importance, the ability to assess the depth of IR in animals before the development of cardiometabolic disorders is of great importance. Based on this, the determination of the index of metabolic disorders in insulin resistance reflects a functional disorder of the cardiovascular system. Its advantages are increased sensitivity to the initial changes in lipid composition, as well as to disorders of glucose metabolism. Analyzing the results of the table, it was found that in horses with insulin resistance, the metabolic index was increased by 82.5% ($p<0.01$) compared to healthy animals (Tahapary, 2022)

Information about the balance between atherogenic and antiatherogenic lipoproteins is provided by an index that reflects the ratio between low-density lipoprotein, LDL, and high-density lipoprotein, HDL, and blood cholesterol (Lonardo, 2005). The atherogenicity index complements the data of the metabolic index and indicates the risk of atherosclerotic plaque formation, which in turn reflects the functional state of the cardiovascular system (Lee, 2021). According to our data, the atherogenicity index in horses with insulin resistance is 54.9% ($p<0.05$) higher than in controls, indicating a high level of atherosclerotic changes.

There is evidence in the literature that the assessment of the ALT/AST ratio reflects the prediction of insulin resistance regardless of comorbidities, and their activity consistently correlated with the above-described insulin resistance indices. The authors found that the ALT/AST ratio is a more useful prognostic marker than HOMA-IR (Han, 2024). As a result of our research on insulin resistance in horses, no such correlation was found, which requires more detailed study.

Conclusion. Thus, in order to assess the development of insulin resistance, appropriate indices have been proposed, the calculation of which is based on data on carbohydrate and lipid metabolism and which complement the data on the pathogenesis of this condition. However, in horses, the choice of a method for studying insulin resistance requires a thorough study.

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ВИВЧЕННЯ ІНТЕГРАЛЬНИХ ІНДЕКСІВ ДІАГНОСТИКИ ІНСУЛІНОРЕЗИСТЕНТНОСТІ У КОНЕЙ

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Резюме. *Оскільки інсулінорезистентність є складовою патогенезу багатьох хвороб у тому числі і розвитку метаболічного синдрому, її виявлення та усунення слід розглядати як важливий і необхідний засіб профілактики захворювань та розвитку їх ускладнень. На відміну від багатьох складних методик основними при виявленні порушення інсулінорезистентності є дані інтегральних індексів. Таким чином метою роботи стало провести діагностику інсулінорезистентності коней з використанням гомеостатичних індексів та встановити їх практичну значимість. Для дослідження було використано 18 коней які були розділені на 2 групи за бальною оцінкою фізичного стану, котра була проведена двома незалежними фахівцями ветеринарної медицини. Розрахунок інтегральних індексів: індекс HOMA-IR, індекс HOMA-β, індекс Caro,* *метаболічний індекс, індекс атерогенності вели згідно літературним даним. Встановлено, що індекс HOMA-β та індекс HOMA-IR були вірогідно підвищені на 18,1% ($p<0,01$) та у 3,7 рази ($p<0,01$) відносно контролю; індекс Caro у коней з інсулінорезистентністю знижено на 39,4% ($p<0,05$); метаболічний індекс підвищено на 82,5% ($p<0,01$); індекс атерогенності у коней з інсулінорезистентністю на 54,9% ($p<0,05$) вищий ніж у контролі. Проте, при вивченні співвідношення АЛТ/АСТ та індекса Matsuda кореляції не встановлено. Таким чином, з метою оцінки розвитку інсулінорезистентності запропоновано відповідні індекси, розрахунок яких оснований на даних вуглеводного та ліпідного обмінів та які доповнюють дані патогенезу цього стану. Проте, у коней вибір методу дослідження інсулінорезистентності потребує досконалого вивчення.*

Ключові слова: інсулінорезистентність, інтегральні індекси, коні, метаболічний синдром, ожиріння.

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