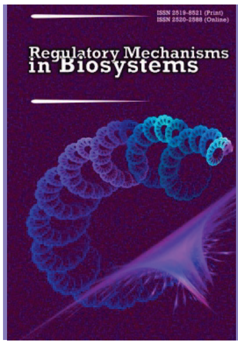

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Diagnostic significance of biochemical parameters of blood serum of ponies in obesity

S. B. Borovkov*, V. S. Boiko*, A. P. Paliy*, V. M. Borovkova**, O. V. Pavlichenko**, I. O. Gerilovich*

*Institute of Experimental and Clinical Veterinary Medicine, Kharkiv, Ukraine
**State Biotechnological University, Kharkiv, Ukraine

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Institute of Experimental and Clinical Veterinary Medicine, Hryhoriia Skovorody st., 83, Kharkiv, 61023, Ukraine. Tel.: +38-050-883-82-92. E-mail: serg_b78@ukr.net

State Biotechnological University, Alchevskikh st., 44, Kharkiv, 61002, Ukraine. Tel.: +38-050-760-62-84. E-mail: vika_borovkova@ukr.net

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Obesity in ponies has become a serious problem leading to the development of metabolic syndrome, insulin resistance and related diseases. The present paper investigates the effect of obesity on metabolic parameters and hormonal status of ponies, especially on the levels of biochemical markers in blood serum. The study was conducted on 18 ponies divided into two groups: 9 clinically healthy animals and 9 obese animals kept in private stables in Kharkiv. All animals received a balanced diet that met their physiological needs, with free access to water and the outdoors. Blood tests included determination of protein and protein fractions, triglycerides, enzyme activity, and hormone levels, including leptin and insulin, and other metabolic markers. The results showed that obese ponies have a decrease in globulin levels, which indicates a decrease in immunity, as well as an increase in albumin concentration, which suggests a violation of the functional state of the liver. Correlations were found between the levels of cholesterol, thyroid hormones (T_3 and T_4), and insulin, indicating a general disruption of the endocrine system. The study confirms that obesity in ponies is associated with increased levels of triglycerides, which are markers of insulin resistance. Biochemical tests, including serum insulin levels, are important in diagnosing metabolic syndrome. Fasting serum insulin concentrations or fasting serum glucose concentrations are simple tools for assessing insulin resistance. The study also suggests that a decrease in triglyceride and high-density lipoprotein levels in obese animals may indicate a disruption in lipid metabolism. High-density lipoproteins provide a reverse pathway for cholesterol to flow from tissues to the liver, where it becomes a source for the formation of bile acids, vitamin D and steroid hormones. Normally, fatty acids, monotriglycerides, and cholesterol are absorbed by intestinal epithelial cells, but this process may be impaired in obese animals. The data obtained emphasize the need for regular monitoring of biochemical parameters in ponies, especially in overweight animals, for early detection of metabolic disorders and prevention of the development of serious diseases. The paper is important for veterinary practice as it provides new data on the impact of obesity on the health of ponies and emphasizes the importance of an integrated approach to their maintenance and treatment.

Keywords: horses; metabolic syndrome; hormones; insulin resistance; dyslipidemia.

Introduction

Obesity is a serious health problem that is common in both humans and animals, including horses. The equine metabolic syndrome, named after the human metabolic syndrome, also has impaired insulin metabolism as a central pathogenic link and is often associated with obesity. Other clinical manifestations of equine metabolic syndrome include cardiovascular changes and adipose tissue dysregulation (Daradics et al., 2021). The term equine metabolic syndrome was first used by Johnson in 2002 as an association of obesity, insulin resistance, and laminitis (Johnson, 2002). The American College of Veterinary Internal Medicine published a consensus statement and included the following factors in the definition of EMS: increased adiposity in specific locations (regional adiposity) or general (obesity), IR, and a predisposition to laminitis. Other conditions included hypertriglyceridemia or dyslipidemia, hyperleptinemia, hypertension, altered reproductive cycle in mares, and elevated systemic inflammatory markers (Frank et al., 2010).

Obesity in horses is a very common pathology (Carslake et al., 2021), ranging from 24% (Jensen et al., 2016) to 54% depending on the region of keeping (Stephenson et al., 2011). A study of obesity in horses and ponies also showed that 45% of 60 horses and ponies of different breeds were overweight (Golding et al., 2023). There is also evidence that although ponies and horses belong to the same species, the prevalence of obesity in them varies considerably – the prevalence of obesity in Scottish ponies was reported to be three times higher than in horses (Potter et al., 2016). The pathogenesis of the metabolic syndrome, especially in the context of horses, involves a complex interaction of several metabolic and endocrine factors, among which insulin resistance and obesity, especially the accumulation of visceral fat, play a key role. In addition, adipose tissue secretes several hormones and inflammatory mediators (adipokines) that can disrupt normal metabolic processes and promote inflammation, further exacerbating insulin resistance.

The predisposition of animals to obesity and insulin resistance is often associated with a state of chronic inflammation. Inflammatory cytokines, such as tumor necrosis factor-alpha and interleukin-6, are elevated in insulin-resistant states and can disrupt insulin signaling pathways. Obesity is also often characterized by abnormal lipid profiles, including elevated triglycerides and low high-density lipoprotein cholesterol. These changes in the lipid profile may further contribute to insulin resistance and increase the risk of cardiovascular disease. Hormonal imbalance of the intermediate pituitary gland may also play a role in the pathogenesis of metabolic syndrome in horses and can lead to elevated cortisol levels, which can increase insulin resistance and contribute to the development of laminitis. It should also be noted that some breeds of horses and ponies may have a genetic predisposition to developing metabolic syndrome and obesity.

The pathogenesis of metabolic syndrome in horses is multifactorial, including insulin resistance, obesity, chronic inflammation, dyslipidemia, hormonal imbalance, nutritional factors, and genetic predisposition. These elements interact to create a metabolic environment that increases the risk of laminitis and other related health problems. Therefore, the study of biochemical parameters of blood serum in animals with metabolic syndrome is a critical issue in modern veterinary medicine.

Horses have transitioned from strenuous labor on farms to participating in sports and recreational activities. As a result, their life expectancy has increased, but they are also more prone to becoming overweight (Ragno et al., 2019). Like other animal species (Bambace et al., 2011), overweight horses show enlarged adipose cells, increased expression of the hormone leptin, and higher levels of pro-inflammatory cytokines, leading to chronic inflammation throughout their bodies (Kanda et al., 2006).

In addition to clinical examination and ultrasonography, biochemical tests play an important role in the diagnosis of metabolic syndrome in horses and ponies. The determination of serum insulin levels is of great diagnostic importance in metabolic syndrome and obesity. Fasting serum insulin concentrations or fasting plasma glucose concentrations are simple tools for assessing animal insulin resistance. However, oral or intravenous glucose and insulin tolerance tests are better for diagnosing metabolic syndrome because they reflect the response to glucose and/or insulin load (Frank et al., 2010). Blood glucose concentrations are measured both at a single time point and over time to assess glucose metabolism in horses in normal and pathological conditions. Several studies have suggested that metabolic syndrome in horses is a factor in the development of laminitis and obesity, but the epidemiology of this condition is poorly described and generally limited to single studies or studies using less sensitive methods (Pollard et al., 2019; Carslake et al., 2021). Small horse breeds are known to be particularly susceptible to metabolic syndrome (Bamford et al., 2014; Norton et al., 2019). Insulin resistance may result in elevated glucose levels or abnormal responses to glucose tolerance tests. A poor response, characterized by elevated insulin and glucose levels, indicates insulin resistance (Ragno et al., 2021). The lipid profile can also be used to assess triglyceride, cholesterol, and other lipid levels. Dyslipidemia, characterized by high triglyceride levels and low high-density lipoprotein cholesterol, is often associated with metabolic syndrome.

Leptin is an adipokine produced by adipose tissue that plays a role in regulating energy balance and insulin sensitivity. Elevated leptin levels may indicate obesity and may be associated with insulin resistance. Furthermore, the expression of the leptin gene was significantly elevated in the adipose tissue of horses with metabolic syndrome, which aligns with other studies that have reported increased plasma leptin concentrations in induced obesity (Bamford et al., 2016). In other animal species, adipocyte volume is the primary determinant of leptin expression in adipose tissue (Guo et al., 2004). This indicator may serve as a marker of adipocyte hypertrophy, rather than simply indicating obesity.

In addition to plasma glucose and serum insulin levels, other metabolic parameters may also be affected by increased obesity in horses. For example, studies have confirmed the association between obesity and serum triacylglyceride concentrations in horses (Frank et al., 2006; Suagee et al., 2013). In line with this, Frank et al. (2010) discovered higher plasma triglyceride levels in obese and insulin-resistant horses than in lean horses without this condition. In contrast, Carter et al. (2009) found no increase in plasma triglycerides or lipids in horses following a significant increase in body weight, despite the animals becoming less sensitive to insulin (Schmidt et al., 2001). Consequently, our objective was to study the biochemical profile in horses and ponies in normal and obese conditions.

Materials and methods

During the experimental studies presented in this paper, all manipulations with the horses involved in the research were carried out taking into account the basic principles of bioethics, according to Article 26 of the Law of Ukraine "On Protection of Animals from Cruelty", 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 ponies kept in private stables in Kharkiv were examined, and two groups were formed. A total of 18 animals were examined: 9 clinically healthy ponies and 9 obese ponies. The research design includes the minimum number of animals for statistical processing by nonparametric methods of statistical analysis, which allows us to establish the informativeness of indicators following the stated aim of the research.

The experimental studies were conducted at the National Scientific Center "Institute of Experimental and Clinical Veterinary Medicine". The animals were provided with adequate nutrition and housing to meet their physiological needs. The animals' diet was balanced in terms of essential nutrients, and all animals had free access to water and were able to walk freely. All animals underwent a general clinical examination under accepted standards. Two independent veterinarians performed Body Condition Scoring (BCS). Blood was taken from the jugular vein on an empty stomach into Vacuette vacuum tubes in an amount of 10 mL for further processing into native blood, plasma, and serum, depending on the research methods, for biochemical study, which was carried out at the Medical Diagnostic Center for Medical Experimental Research in Kharkiv.

The laboratory has all necessary licenses and is a certified laboratory according to the Certificate of Conformity of Measurement Systems according to DSTU ISO 10012:2005. The serum was analyzed using a COBAS C 311 photometric system (manufactured by Roche Diagnostics GmbH, Germany) with ion-selective electrodes for the study of clinical and biochemical parameters of serum. The content of total protein and protein fractions (albumin and globulin fractions: α_1, α_2, β, γ), activity of the enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and gamma-glutamyl transferase (GGT), hormone content in the blood serum were determined: insulin, leptin, triiodothyronine (T_3), thyroxine (T_4) and thyroid-stimulating hormone (TSH), total cholesterol, triglycerides, high-density lipoprotein (HDL), low-density lipoprotein (LDL) and very-low-density lipoprotein (VLDL).

Leptin was determined by enzyme-linked immunosorbent assay. In the first step, leptin binds to the immobilized antibody, followed by the addition of streptavidin-HRP and the addition of an enzyme substrate of blue color. Total protein was determined by the biuret method with the formation of a purple-colored complex. The determination of protein fractions was performed by the method based on the fact that phosphate solutions of a certain concentration precipitate different protein fractions of blood serum to form a suspension. ALT, AST, and GGT were determined by the enzymatic colorimetric method. Glucose was determined by a standard enzymatic method using hexokinase. Triiodothyronine (T_3), thyroxine (T_4), and thyroid-stimulating hormone (TSH) concentrations and insulin levels were determined by electrochemiluminescence immunoassay (ECLIA). Total cholesterol was determined by the enzymatic calorimetric method, the principle of which is that cholesterol esters are broken down into cholesterol and fatty acids by cholesterol esterase so that cholesterol esterase catalyzes the oxidation of cholesterol to cholest-4-en-3-one and hydrogen peroxide. Lipoprotein cholesterol was determined by the homogeneous calorimetric method, which is based on the principle of using cholesterol esterase and cholesterol oxidase in the presence of surfactants that selectively absorb certain types of lipoproteins. The tables show Median, Q_1, and Q_3 quartiles; a significant difference between groups was determined based on the calculation of the Mann-Whitney test ($P < 0.05$), and Spearman's rank correlation coefficient was also calculated.

Results

Protein molecules play an important role in regulating metabolism. They are found in enzymes, hormones, and other biologically active substances. The results of their metabolism in clinically healthy animals and obese ponies are shown in the Table 1. It was found that the level of total protein remained unchanged, but its fractions underwent significant changes. Thus, in ponies with signs of obesity, there is an increase in albumin levels by 5.5% ($P < 0.01$) compared to clinically healthy animals. According to the results of the content of globulin fractions, a decrease in α_1 globulin and α_2 globulin by 0.4% and 2.4% ($P < 0.01$), β globulins by 2.5% ($P < 0.05$), and a decrease in γ globulins (difference was 0.5%) is manifested only within the tendency in the blood serum of ponies with signs of obesity. A correlation was observed between α_1 globulin and γ globulin levels ($r = 0.741 \pm 0.062$; $P = 0.014$); between α_1 globulin and TSH levels ($r = 0.640 \pm 0.055$; $P = 0.046$), and between β globulin and TSH levels ($r = 0.716 \pm 0.042$; $P = 0.020$).

Table 1
Indicators of the serum protein profile of ponies in normal and obese conditions (n = 9)

No. of the group	Total protein, g/L	Albumin, %	α_1, %	α_2, %	β, %	γ, %
1. Mean ± SD	65.1 ± 2.1	38.7 ± 1.3	4.9 ± 0.2	8.8 ± 0.3	23.7 ± 0.7	24.9 ± 0.8
Q_1–Q_3	63.3–67.2	37.2–39.9	4.8–5.0	8.5–9.1	23.2–24.2	24.3–25.5
2. Mean ± SD	66.3 ± 2.4	44.2 ± 1.5**	4.5 ± 0.1	6.4 ± 0.2**	21.3 ± 0.6	23.6 ± 0.7
Q_1–Q_3	64.4–67.8	42.2–45.3	4.4–4.6	6.1–6.5	20.1–21.9	22.9–24.3

Note: ** P < 0.01, compared between groups.

The primary pathogenic link in the metabolic syndrome in obese horses is insulin resistance and hormonal status disorders. Table 2 provides data on the dynamics of these indicators. The data demonstrate a 47.1% (P < 0.001) increase in glucose levels in animals with signs of obesity, indicating a general dysfunction of the body's endocrine system. This dysfunction may result from a deficiency of insulin, a hormone produced by the pancreas, or from the inability of the liver and body tissues to properly process and absorb glucose. The results indicated a negative correlation between glucose concentration and several other variables, including very-low-density lipid levels (r = 0.775 ± 0.088; P = 0.008), AST activity (r = 0.772 ± 0.065; P = 0.003), and alkaline phosphatase activity (r = 0.732 ± 0.071; P = 0.016). Our studies have shown that peripheral hormonal signals such as leptin are reduced by 42.9% (P < 0.001) in ponies with signs of obesity, reflecting an imbalance in energy balance due to excessive feed intake, impaired fatty acid and glucose metabolism, and insulin regulation. It is also important to note the negative correlation between leptin and low-density lipoprotein levels (r = 0.633 ± 0.043; P = 0.049) and the positive correlation between leptin and ALT activity (r = 0.852 ± 0.073; P = 0.002) and glucose levels (r = 0.670 ± 0.056; P = 0.034). Thus, the data obtained revealed a violation of the adipo-insulin axis, which determines the interaction between insulin and leptin to control glucose production.

Dyslipidemia in the context of obesity may be a diagnostic criterion for animal health and may have a prognostic character for the course of this pathology (Table 3).

Table 2
Indicators of hormonal status of pony blood serum in norm and metabolic syndrome (n = 9)

No. of the group	Insulin, μIU/mL	Leptin, ng/mL	Glucose, mmol/L	Glycated hemoglobin, %
1. Mean ± SD	0.728 ± 0.031	0.865 ± 0.0292	3.970 ± 0.117	66.07 ± 2.15
Q_1–Q_3	0.696–0.758	0.835–0.887	3.88–4.08	64.38–68.29
2. Mean ± SD	3.875 ± 0.149***	0.494 ± 0.017***	5.840 ± 0.195***	66.68 ± 2.06
Q_1– Q_3	3.748–4.005	0.480–0.510	5.63–6.03	64.92–68.36

Note: *** P < 0.001 compared between groups.

Table 3
Indicators of the ponies' serum lipidogram in norm and metabolic syndrome (n = 9)

No. of the group	Total cholesterol, mmol/L	Triacylglycerols, mmol/L	High-density lipoprotein, mmol/L	Low-density lipoprotein, mmol/L	Very-low-density lipoprotein, mmol/L
1. Mean ± SD	1.81 ± 0.05	0.32 ± 0.01	1.31 ± 0.04	0.34 ± 0.02	0.15 ± 0.01
Q_1–Q_3	1.79–1.84	0.30–0.33	1.27–1.35	0.31–0.35	0.14–0.16
2. Mean ± SD	2.06 ± 0.09**	0.35 ± 0.01**	1.24 ± 0.04	0.68 ± 0.02***	0.36 ± 0.01***
Q_1–Q_3	2.17–2.34	0.36–0.38	1.22–1.26	0.67–0.69	0.32–0.39

Note: ** P < 0.01, *** P < 0.001 compared between groups.

Analyzing the results of the studies (Table 3), in ponies with signs of obesity, the content of total cholesterol in the blood serum increased by 13.8% (P < 0.01) compared with clinically healthy animals, which is due to low-density lipoprotein (LDL), which has an increase of 2.0 times (P < 0.001), which amounted to 32.8% of total cholesterol. There was a correlation between cholesterol and T_4 (r = 0.680 ± 0.046; P = 0.030); high-density lipoprotein (HDL) level and A/G ratio (r = 0.721 ± 0.057; P = 0.019) and LDL and alkaline phosphatase activity (r = 0.631 ± 0.055; P = 0.042). Normally, in the blood of animals, lipoproteins of this fraction transport cholesterol to tissues, and their level is insignificant, but this indicator significantly depends on the type of diet and the consumption of food containing fats. An increase in the concentration of cholesterol of this particular fraction in obesity is a pathogenic factor in the development of internal diseases in animals and indicates the existence of metabolic syndrome. Also, with elevated total cholesterol and low-density lipoprotein levels, animals with signs of obesity showed a 9.4% increase in triglycerol levels (P < 0.01) and a nearly 2.5-fold increase in very-low-density lipoprotein (VLDL) levels (P < 0.001). We believe this is because high-density lipoproteins provide a reverse pathway for cholesterol from tissues to enter the liver, where it becomes a source for the formation of bile acids, vitamin D, and steroid hormones.

According to the results of our studies (Table 4), in ponies with signs of obesity, serum ALT activity increases significantly by 11.9% (P < 0.01) compared to clinically healthy animals, and AST activity decreases by 15.7% (P < 0.001).

Table 4
Indicators of the enzyme profile of pony blood serum in norm and metabolic syndrome (n = 9)

No. of the group	Alanine aminotransferase, U/L	Aspartate aminotransferase, U/L	Alkaline phosphatase, U/L	Gamma-glutamyl transferase, U/L
1. Mean ± SD	10.9 ± 0.4	384.0 ± 12.9	403.2 ± 9.0	35.7 ± 1.1
Q_1–Q_3	10.6–11.2	373.6–390.4	396.9–409.6	35.0–36.5
2. Mean ± SD	12.2 ± 0.3**	323.8 ± 9.2***	138.6 ± 4.3***	14.7 ± 0.3***
Q_1–Q_3	11.9–12.4	315.2–334.6	134.7–142.7	14.6–14.9

Note: ** P < 0.01, *** P < 0.001 compared between groups.

With regard to the function of alkaline phosphatase, which catalyzes the hydrolysis of phosphoric acid ethers, this enzyme reflects not only the condition of the liver but also the biliary tract and the process of bile formation. Bile is produced in the liver from blood cells and this process is continuous, but it enters the intestines only after a meal. The decrease in alkaline phosphatase activity by 65.6% (P < 0.001) in ponies with signs of obesity may be associated with impaired bile formation due to changes in the mechanism of hepatic secretion and vitamin B6 deficiency. There was a correlation between AST activity and alkaline phosphatase activity (r = 0.743 ± 0.076; P = 0.014). The dynamics of the enzyme composition is complemented by the activity of the enzyme gamma-glutamyl transpeptidase, an enzyme involved in the metabolism of amino acids. It catalyzes the transfer of a gamma-glutamyl residue from a gamma-glutamyl peptide to an amino acid, another peptide, or, during hydrolysis, to water. This enzyme is found in the cell membranes of many tissues, including liver, kidney, and pancreas. It is also found in other tissues, such as the intestines, spleen, heart, and brain, but at lower levels. The highest concentration of the enzyme is found in the kidneys, but the liver is considered the source of normal enzyme activity, so any fluctuations in GGT are primarily indicative of liver problems. In ponies showing signs of obesity, GGT levels are reduced by a factor of 2.4 ($P < 0.001$), probably indicating metabolically related fatty liver disease and impaired bile formation. The status of the hormones produced by the thyroid gland significantly affects the development of obesity. Thus, according to the results presented in Table 5, it was found that the levels of T_3 and T_4 hormones were increased by 33.1% ($P < 0.001$) and 18.3% ($P < 0.01$), respectively, in ponies with signs of obesity compared to healthy animals. There was no difference in thyroid-stimulating hormone levels between the groups of ponies. Thus, in obesity, the increase in T_4 and T_3 levels and normal TSH is due to a decreased level of leptin, an important neuroendocrine regulator of the hypothalamic-pituitary-thyroid axis, which inadequately regulates the expression of the thyrolyberine gene in the paraventricular nuclei of the hypothalamus.

Table 5
Indicators of the hormonal status of pony blood serum in norm and metabolic syndrome

No. of the group	Triiodothyronine, pmol/L	Thyroxine, pmol/L	Thyroid-stimulating hormone, ng/mL
1. Mean ± SD	3.02 ± 0.11	9.42 ± 0.17	0.51 ± 0.08
Q_1–Q_3	2.94–3.11	9.34–9.55	0.42–0.58
2. Mean ± SD	4.02 ± 0.08***	11.14 ± 0.35**	0.50 ± 0.09
Q_1–Q_3	3.96–4.08	10.77–11.43	0.40–0.62

Note: ** $P < 0.01$, *** $P < 0.001$ compared between groups.

Discussion

Proteins are high-molecular-weight organic nitrogen-containing compounds that play a crucial role in all life processes and phenomena (Paliy et al., 2024). They occupy a central place in the structure of living matter and play a primary role in its functioning. The major fractions are albumin, globulin fractions (alpha 1, alpha 2, beta, and gamma globulins), and fibrinogen (Garg et al., 2020; Alexy et al., 2022).

The α_1-globulin and α_2-globulin fractions are characterized by a significant content of carbohydrates, with a predominance of hexoses, somewhat less of hexosamines, and even less of sialic acids and fructose. These fractions inhibit many proteolytic enzymes and perform transport functions (transport of lipids, thyroxine, corticosteroid hormones, copper ions, and hemoglobin). The B globulin fraction consists of various proteins (transferrin, hemopexin, complement components, and some immunoglobulins), including lipoproteins. This fraction is involved in transporting lipids, ferric and heme ions, and immune reactions as antitoxins. The γ-globulin fraction consists of immunoglobulins (in descending order of abundance – IgG, IgA, IgM, IgE), which functionally represent antibodies that provide humoral immune defense against infections and foreign substances (El-Khateeb et al., 2020; Korkh et al., 2024).

The albumin fraction performs an important transport function – they are involved in the transfer of fatty acids, lipids, bilirubin, aminoacids, metal ions, and regulate the content of hormones, and their inadequate amount leads to disorders of these functions, which primarily affects the functional state of the liver (Gnacińska et al., 2010; Romanko et al., 2023).

Thus, our studies are consistent with the research of other scientists indicating a decrease in the immunity of obese animals, as the level of globulins reflects the morphological maturity and functional integrity of immunoreactive tissue (El-Khateeb et al., 2020).

The albumin-globulin ratio (A/G) is a complex indicator of the overall activity of catabolism and anabolism, which is used to characterize protein metabolism. A reduction in the albumin-globulin ratio is a common occurrence in many pathological conditions. This is associated with an increase in the globulin fraction and a decrease in the amount of albumin. Conversely, an increase in the ratio is rarely observed, particularly when anabolic processes predominate over catabolic ones. Our studies demonstrate that the A/G ratio increased by 24.3% ($P < 0.01$) in ponies with obesity (Wei et al., 2022).

As with other animals, protein metabolism in ponies is dependent on carbohydrate metabolism. This is because the tricarboxylic acid cycle (TCA) is a link between many metabolic processes, including protein and carbohydrate metabolism. The TCA is a crucial point in the metabolic process, where glycolysis and the oxidative breakdown of carbohydrates (pyruvic, ketoglutaric, and oxalic acids) form amino acids through amination and transamination. These amino acids are then utilized for protein synthesis (Antonyshyn, 2015).

In the human and animal body, the liver plays a significant role in carbohydrate metabolism, where glycogen, a reserve form of glucose, is stored. The liver is responsible for the constant synthesis and breakdown of glycogen, which ensures optimal blood glucose levels. Free glucose enters the bloodstream and is utilized by other organs and tissues. The formation of oligos and polysaccharides requires the presence of active monosaccharides, including nucleotides linked to sugars, which are essential for biochemical reactions. It should be noted that glycogen is also accumulated in skeletal muscle, in addition to the liver. It ensures that these tissues have sufficient energy and a consistent level of glucose (liver) between meals (Di Gregorio et al., 2021; Khadka, 2022).

The pancreatic hormones insulin and glucagon, as well as the adrenal medulla hormone adrenaline, play an important regulatory role in maintaining physiological blood glucose levels. Insulin, the β hormone of the pancreatic islet tissue, is an antagonist of adrenaline and glucagon. Insulin promotes the synthesis of glycogen in the liver and muscles, the oxidation of monosaccharides and their conversion to fats, and stimulates the synthesis of leptin in adipocytes (Kahn et al., 2019).

As outlined in the literature, hyperinsulinemia is observed in the blood when there is an increase in the secretion of the hormone β by cells in the pancreatic islets. This occurs when cells in insulin-dependent tissues (mainly adipocytes, myocytes, and hepatocytes) respond less to this hormone due to the accumulation of fat inside them due to excess energy reserves. This, in turn, leads to the development of insulin resistance (Zak et al., 2020). This finding is reflected in our results, which indicate a 5.3-fold increase in insulin levels in ponies with obesity-related symptoms.

Since insulin and leptin have hormonal feedback, the so-called adipo-insulin axis, which causes an interaction between these hormones to control appetite and glucose production, the study of leptin dynamics will reflect the state of balance between fat reserves and food consumption (Abo El Maaty et al., 2020). In horses, leptin is synthesized only by white adipose tissue cells and enters the peripheral bloodstream in proportion to the percentage of body fat (Kędzierski, 2014; La Sala & Pontiroli, 2020). Normally, it increases the sensitivity of tissues to insulin, blocking its synthesis and secretion by β-cells of the pancreas. Summarizing the functions of leptin, the main ones are as follows: it inhibits the synthesis of neuropeptide Y; increases the production of glucagon-like peptide 1 (GLP 1); is an antisteatogenic hormone, i.e. prevents the development of glucotoxicity, regulates intracellular fatty acid homeostasis, preventing the development of lipotoxicosis; induces puberty, gonadal development, normalization of gonadotropin secretion; promotes Na excretion and K retention; influences T-cell immunity (Lee et al., 2013; Dempersmier & Sul, 2015).

Publications on insulin resistance and energy metabolism disorders indicate that animals with such metabolic disorders are prone to hyperlipidemia (Daradics et al., 2021). Therefore, there is an urgent need to study the characteristics of lipid metabolism disorders and to determine the validity of their indicators in the blood serum of ponies with signs of obesity.

Lipids are part of cell membranes and are the main source of energy, solvents of vitamins A, D, E, and K, participate in the synthesis of steroid hormones, create a thermally insulating and water-repellent layer in surface tissues, and play a mechanical protective role (capsula adiposa renis). Lipid metabolism disorders lead to changes in their functions and the development of pathological processes such as obesity, wasting, dyslipoproteinemia, lipodystrophy, and lipidosis (Poznyak et al., 2020).

However, unlike other animal species, ponies do not develop atherosclerotic processes because, according to Norton et al. (2019), the majority of cholesterol is found in high-density lipoproteins.

In clinically healthy ponies, as shown in the table, the composition of total cholesterol is as follows: the proportion of high-density lipoprotein cholesterol is 72.5%, low-density lipoprotein cholesterol is 18.5%, and very-low-density lipoprotein cholesterol is 8.2%, which is in compliance with Kaneko et al. (2008).

Normally, fatty acids, monotriglycerides, and cholesterol are absorbed by intestinal epithelial cells. They are then attached to apoB48 (the intestinal form of apoB), apoAI, and apoAIV, which coalesce into large particles called chylomicrons. On their way from the intestine to the liver, chylomicrons bind to apoCII (cofactor) and, with the help of the enzyme lipoprotein lipase, lose 90% of the triglycerides that have accumulated in adipose and muscle tissue. Residual chylomicrons, with the help of apoE, high-density lipoproteins interact with liver receptors and are taken up by hepatocytes, where they and endogenous lipids form very-low-density lipoproteins, which are also enriched in triglycerides (Heliczer et al., 2017; Santos-Baez & Ginsberg, 2020). Based on the above, the dynamics of cholesterol and triglycerides in ponies with signs of obesity indicate a violation of fatty acid metabolism in liver tissue.

The dominant role in laboratory diagnosis of liver diseases is played by determining the activity of enzymes synthesized by hepatocytes and biliary epithelial cells. The largest group of diagnostically important enzymes are indicator enzymes, which include ALT, AST, and excretory enzymes of alkaline phosphatase.

Transaminases catalyze the reactions that transfer an α-amino group from an amino acid to the α-carbon atom of an α-keto acid, resulting in the formation of an α-keto analog of the original amino acid and a new amino acid. The coenzyme in transamination reactions is pyridoxal phosphate, a derivative of vitamin B_6. The most important diagnostic values among the aminotransferases are alanine aminotransferase (ALT) and aspartate aminotransferase (AST). AST catalyzes the reverse reaction of amino group transfer from aspartic acid to α-ketoglutarate to form oxaloacetate and glutamic acid. ALT is an enzyme that catalyzes the transfer of an amino group from alanine to α-ketoglutarate to form pyruvate and glutamic acid. Transamination reactions are universal to all tissues but are most abundant in the liver (Kaneko et al., 2008).

According to scientific research, thyroid hormones are also involved in the regulation of energy metabolism, which is impaired in obesity (Singh et al., 2018). T_3 controls energy expenditure through central and peripheral pathways, stimulating specific neurons in the ventromedial nucleus that activate the sympathetic nervous system, which in turn innervates brown adipose tissue and leads to adaptive thermogenesis (Gereben et al., 2008; Ortiga-Carvalho et al., 2016); at the same time, T_3 acts directly in brown adipose tissue and activates the thermogenic program by controlling lipid metabolism and uncoupling protein 1 activation (Obregon, 2014).

Thyroxine (T_4) is the major hormone secreted by the equine thyroid gland. Only free fractions of T_4 are metabolically active. It can be deiodized in target tissues by deiodinases (type 1 and 2) to form the more metabolically active T_3 (Frank et al., 2006; Breuhaus, 2011). Another deiodinase (type 3) degrades T_4 to reverse T_3 (rT_3), which has low metabolic activity (Cheng et al., 2010; Costello et al., 2019).

Thyroid stimulating hormone (TSH) and thyrolyberine levels are also critical determinants of energy metabolism throughout the body. They exert thyroid and non-thyroid effects, combining signals from the nutritional state and the adrenergic nervous system with the fine regulation of iodothyronine production (Costello et al., 2019, Yuzvenko, 2021).

Conclusion

Our studies of protein metabolism indicate a decrease in immunity in obese animals, since a decreased level of globulins reflects the morphological maturity and functional completeness of immunoreactive tissues, an increased amount of albumin indicates a violation of the functional state of the liver, and an increase in the albumin/globulin ratio indicates the predominance of anabolic over catabolic processes.

Our data reflect a general dysfunction of the body's endocrine system and the inability of the liver and body tissues to properly process and assimilate glucose, which is based on leptin deficiency and increased concentrations of T_3 and T_4, insulin, and glucose in the blood serum of ponies with signs of obesity. The lipidogram of blood serum of ponies with signs of obesity is characterized by hypercholesterolemia due to an increase in the content of low-density lipoprotein cholesterol compared to clinically healthy animals against the background of a decrease in the content of triacylglycerols, very low and high-density lipoproteins, indicating a violation of fatty acid metabolism in liver tissue.

The decrease in the activity of alkaline phosphatase, gamma-glutamyltransferase, and AST, as well as the increase in ALT activity in ponies with signs of obesity, was found to be associated with impaired bile formation due to changes in the mechanism of hepatic secretion.

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