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# USING BIOPELLETS WITH METFORMIN IN THE EXPERIMENTAL METABOLIC SYNDROME

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The global prevalence of metabolic syndrome in Ukraine and the world gives reason to consider it one of the most dangerous diseases of the modern world. One of the promising directions in the pharmacotherapy of pathological conditions is the use of the drug in the form of bioidentical implants that contain a medicinal substance. Experimental metabolic syndrome in animals was modeled with the help of a Cafeteria diet, which imitated the problematic eating patterns of modern humans. Animal body weight, blood glucose, lactate and insulin content, HOMA index, and lipid metabolism indicators were evaluated as markers of metabolic syndrome and criterion signs of treatment effectiveness. Results showed that a 6-week Cafeteria diet causes a metabolic imbalance and is accompanied by signs characteristic of metabolic syndrome. Probable signs of insulin resistance accompanied these changes. Both the tablet and pellet form of the drug positively affected the indicators of carbohydrate metabolism and reduced the manifestations of insulin resistance. Metformin in bioidentical implants also significantly improved experimental animals' general condition and appearance.

**Key words**: metabolic syndrome, metformin, laboratory animals, lipid spectrum, hyperglycemia, insulin resistance, pellets.

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# ЗАСТОСУВАННЯ БІОПЕЛЛІЄТ З МЕТФОРМІНОМ ЗА ЕКСПЕРИМЕНТАЛЬНОГО МЕТАБОЛІЧНОГО СИНДРОМУ

Глобальна розповсюдженість метаболічного синдрому в Україні та світі дає підстави вважати його одним з найбільш небезпечних захворювань сучасного світу. Одним із перспективних напрямків в фармакотерапії патологічних станів є використання препарату у вигляді біоідентичних імплантів, які містять лікарську речовину. Експериментальний метаболічний синдром у тварин моделювали за допомогою кафе-дієти, яка імітувала моделі проблемного харчування сучасної людини. В якості маркерів метаболічного синдрому та критеріальних ознак ефективності лікування оцінювали масу тіла тварин, вміст глюкози, лактату та інсуліну в крові, індекс HOMA, показники ліпідного обміну. Результати показали, що 6-тижневе харчування кафе-дієтою викликає метаболічний дисбаланс і супроводжується ознаками, характерними для метаболічного синдрому. Дані зміни супроводжувались вірогідними ознаками інсулінорезистентності. Як таблетована, так і пелетована форма препарату позитивно впливали на показники метаболізму вуглеводів та зменшували прояви інсулінорезистетності. Метформін у вигляді біоідентичних імплантів крім того значно покращував загальний стан та зовнішній вигляд експериментальних тварин.

**Ключові слова**: метаболічний синдром, метформін, лабораторні тварини, ліпідний спектр, гіперглікемія, інсулінорезистентність, пеллети.

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Metabolic syndrome, according to the WHO definition, is a complex pathological condition characterized by abdominal obesity, insulin resistance, hypertension, and hyperlipidemia. The prevalence of metabolic syndrome in the world and in Ukraine gives reason to consider it one of the most dangerous non-infectious diseases of the modern world. Metabolic syndrome is associated with a significant risk of developing type 2 diabetes, cardiovascular disease, and strokes, which in turn cause significant financial costs in the health care system and often lead to disability of patients. The total cost of the disease, including health care costs and loss of potential economic activity, is estimated in trillions [12]. Central features of the metabolic syndrome are insulin resistance, visceral obesity, atherogenic dyslipidemia, and endothelial dysfunction. These conditions are interrelated and share common mediators, pathways, and pathophysiological mechanisms.

As a clinical object, scientists have relatively recently focused on metabolic syndrome, but the amount of research devoted to its study is growing exponentially every year [5]. However, many aspects of this clinical entity are still not fully understood, and many questions remain unanswered.

Among the drugs traditionally indicated in the treatment of both type 2 diabetes and metabolic syndrome are biguanides, of which metformin is the most popular. It also exhibits anti-atherogenic properties, reduces cholesterol synthesis in the liver, lowers the level of low-density lipoproteins, and increases the content of high-density lipoproteins. Also, the drug has an endotheliotropic effect, a decrease

in the activity of inflammation in blood vessels, and an antithrombotic effect, which is a consequence of an increase in the activity of endothelial NO-synthase and a decrease in the production of angiotensin II. Metformin is considered the "gold standard" among insulin sensitizers, widely used in treating insulin resistance in polycystic ovary syndrome. The antiviral effect of metformin has been described, and it can occur through several mechanisms, including blocking the angiotensin-converting enzyme 2 receptor, as well as anticancer and immunoregulatory impact [11]. These potential mechanisms of action of metformin are promising in various clinical situations, such as inflammatory diseases, autoimmune diseases, cancer, and coronavirus disease [6].

Despite the vast experience of using metformin as a hypoglycemic agent, the patient's compliance with the use of a sufficiently large tablet began to decrease somewhat. The presence of comorbid pathology in patients usually requires taking a large number of drugs, which can lead to problems of compatibility of the used means. Antidiabetic drugs must be taken at the appropriate time to ensure a stable blood sugar level, which is not always convenient for the patient. In addition, metformin can cause side effects, including gastrointestinal disorders, hepatotoxicity, lactic acidosis, etc. [4, 9, 14, 15].

Therefore, a new form of metformin appeared in the pharmaceutical market. "Glucophage XR" (extended-release) was invented specifically for those patients who cannot tolerate the usual forms of metformin due to certain undesirable phenomena from the gastrointestinal tract. Glucophage XR has significantly better tolerability, increasing patient treatment adherence [2].

Another promising field is the use of the drug in bioidentical implants (pellets) that contain a medicinal substance. The capsule is administered subcutaneously, and for about 4–6 months, the drug is gradually and uniformly distributed in the body. The use of medicinal products in the form of pellets began in the 30s of the 20th century, and work on improving this medicinal form continues up to this day [13]. This way of using drugs, including metformin, is becoming more and more popular. Still, there are practically no results of targeted comparative studies of the efficacy and safety of the drug using such administration methods.

**The purpose** of the study was to evaluate the efficacy and safety of metformin pellets in terms of insulin resistance, carbohydrate, and lipid metabolism in animals with experimental metabolic syndrome.

**Materials and methods.** The study was performed on 28 male Syrian hamsters weighing 90–120 g housed in the vivarium of the National Pirogov Memorial Medical University, Vinnytsia. The study was performed in spring and started in the morning. All stages of the study were carried out in compliance with international requirements for the Humane Treatment of Animals, following the rules of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" and current laws of Ukraine and approved by the Bioethics Commission of National Pirogov Memorial Medical University, Vinnytsia (Protocol No. 2 dated March 2, 2024). The animals were kept in standard vivarium conditions, with a 12-hour day/night regime. Experimental metabolic syndrome in animals was modeled with the help of a Cafeteria (CAF) diet, which imitated the problematic eating patterns of modern humans [7]. According to the literature, the CAF diet mixture consisted of "Melted milk" cookies, potato chips – 45 %; cream margarine (source of fats) – 40 %; soy goulash (source of protein) – 10 %; vegetables (carrots, cucumbers, cabbage, etc.) – 5 %. A 10 % fructose solution replaced drinking water. Animal's access to food and drink was ad libitum. The simulation duration was 6 weeks (42 days). The treatment period began with the 7th week of the experiment.

According to the purpose, the experimental animals were divided into groups of 7 individuals each. The 1st group was the control group (hamsters that received a standard diet according to species norms). Metabolic syndrome was reproduced in animals of groups 2–4 (experimental). Subsequently, the animals of the 2nd experimental group continued to eat the CAF diet without correction. Animals of the 3rd group with the background of metabolic syndrome were subcutaneously implanted with a metformin pellet (Biopell Medical, USA, at 200 mg/kg). Animals of the 4th group received similar doses of metformin orally (Metformin Sandoz) as a suspension through an intragastric tube once a day for 30 days.

Animal body weight, blood glucose, lactate and insulin content, HOMA index, and lipid metabolism indices were evaluated as markers of metabolic syndrome and criterion signs of treatment efficacy [3]. Whole blood, blood serum, and liver hydrolysate were used for research. Serum was obtained by centrifuging whole venous blood at 1500 rpm for 20 min. The liver hydrolysate was obtained by tissue desmolysis using a 30 % potassium hydroxide solution. Glucose content in peripheral blood was assessed using an Accu-Chek Active glucometer (Rouche Group, Germany). The Seifter test determined glycogen level in the liver hydrolysate: glycogen was precipitated with ethanol, then hydrolyzed in a medium of concentrated sulfuric acid, and the increase in glucose was determined [1]. Serum insulin levels were assessed using an enzyme-linked immunosorbent assay using the Insulin ELISA Kit (Elabscience

Biotechnology Inc., USA) according to the manufacturer's instructions on the STAT-FAX 303+ analyzer (US) at a wavelength of 450 nm (differential filter – 630 nm). The index of insulin resistance (HOMA-IR) was determined according to the well-known formula:

HOMA-IR = fasting glucose (mmol/L) X fasting insulin (mU/L)/22.5

The lactate content, total cholesterol, and triglycerides in blood serum were determined using a standard set of reagents produced by "Filisit-Diagnostika" (Ukraine). Statistical processing of the obtained results was performed using standard methods using the "Statistica SPSS 10.0 for Windows" program (license No. 305147890). The obtained results were presented in the form of M±m. The Shapiro-Wilk test was used to check the normality of the distribution. The reliability assessment of the difference in mean parameters was carried out using the Student's T-test (for a normal distribution) and the Mann-Whitney U-test (if the distribution deviates from the normal). Differences at $p<0.05$ were considered statistically significant.

**Results of the study and their discussion.** Before starting the study, basal glycemic levels were determined to exclude animals with spontaneous hyperglycemia; during the experiment, after 1 week of using the CAF diet, glycemic levels were monitored, excluding animals with fasting glucose levels above 6.5 mmol/L. Our results showed that a 6-week CAF diet causes a metabolic imbalance and is accompanied by signs characteristic of metabolic syndrome. Thus, the mean body weight of hamsters on the 40th day of the experiment probably increased by 1.36 times ($p<0.05$) compared to the initial data. It was accompanied by an increase in glycemia by 92.3 % and disturbances in carbohydrate metabolism – a decrease in the content of liver glycogen (by 56.1 %) and lactic acidosis (an increase in the blood lactate level by 43.3 % compared to the indices of the control group). These changes were accompanied by probable signs of insulin resistance – an increase in the content of insulin in the blood (by 94.0 %) and an increase in the HOMA IR index by 3.75 times ($p<0.05$), respectively)). Dyslipidemia was also noted in the group of control pathology animals compared to intact animals. An increase in total cholesterol by 96.7 % and triglycerides by 84.1 % ($p<0.05$) was observed in blood serum (Table 1–2).

Table 1

**Parameters of carbohydrate metabolism during experimental metabolic syndrome and against the background of metformin pellets (M±m)**

| Animal groups | Biochemical parameters | | |
|---|---|---|---|
| | Blood glucose concentration, mmol/L | Liver glycogen, mg/g of tissue | Blood lactate concentration, mmol/L |
| Control group | 5.46±0.44 | 23.0±0.89 | 7.12±0.54 |
| Metabolic syndrome without correction | 10.5±0.59* | 10.1±0.61* | 10.2±0.63* |
| Metabolic syndrome + metformin pellets | 6.67±0.50# | 21.4±0.83# | 13.9±0.69*# |
| Metabolic syndrome + metformin tablets | 6.31±0.46# | 20.9±0.80# | 16.8±0.74*#& |

Notes: 1. * – $p<0.05$ relative to the control group. 2. # – $p<0.05$ relative to the untreated animals with metabolic syndrome group. 3. & – $p<0.05$ relative to the group of animals with metabolic syndrome treated with metformin pellets.

Table 2

**Parameters of insulin resistance and lipid metabolism in the experimental metabolic syndrome and against the background of metformin pellets (M±m)**

| Animal groups | Biochemical parameters | | | |
|---|---|---|---|---|
| | Blood insulin, mIU/L | HOMA-IR | Total cholesterol, mmol/L | Triglycerides, mmol/L |
| Control group | 15.2±0.53 | 3.70±0.27 | 2.46±0.12 | 0.95±0.04 |
| Metabolic syndrome without correction | 29.5±0.76* | 13.9±0.62* | 4.84±0.23* | 1.75±0.05* |
| Metabolic syndrome + metformin pellets | 17.3±0.62*# | 5.15±0.39*# | 2.70±0.13# | 1.03±0.04# |
| Metabolic syndrome + metformin tablets | 16.9±0.58*# | 4.78±0.35*# | 2.60±0.11# | 0.98±0.03# |

Notes: 1. * – $p<0.05$ relative to the control group. 2. # – $p<0.05$ relative to the untreated animals with metabolic syndrome group. 3. & – $p<0.05$ relative to the group of animals with metabolic syndrome treated with metformin pellets.

Treatment with metformin significantly reduced the manifestations of metabolic syndrome in hamsters. Both the tablet and pellet form of the drug during the treatment period positively affected the indices of carbohydrate metabolism. They contributed to reducing blood glucose content (by 39.9 and 36.5 %, respectively ($p<0.05$), increased tissue sensitivity to insulin, and decreased manifestations of insulin resistance. Thus, under the conditions of using metformin in pellets, the blood insulin level probably decreased by 41.4 %, and the HOMA-IR index declined by 2.69 times compared to untreated

animals. The degree of changes in similar indices in the group of animals that received metformin orally was comparable and did not differ statistically. The resulting changes in biochemical indices are logical and related to biguanide's primary mechanisms of action. Thus, according to the literature, intracellular metformin also inhibits the Mitochondrial Respiratory Complex I, accompanied by a decrease in ATP synthesis and AMP accumulation, and a reduction in the activity of gluconeogenesis, fatty acid, and cholesterol synthesis. The decrease in the gluconeogenesis activity in the liver, induced by metformin, is explained not only by energy deficit [6] but also by the blocking of lactate transport into hepatocytes, inhibition of the main enzymes of gluconeogenesis: pyruvate carboxylase, phosphoenolpyruvate carboxykinase, and glucose-6-phosphatase. Due to the activation of the second substrate of the insulin receptor (IRS-2), metformin enhances glucose transport into hepatocytes, mediated by the GLUT-1 transporter. In the liver, metformin also affects the synthesis and catabolism of lipids. In adipocytes, this drug activates lipolysis and β-oxidation of fatty acids; therefore, their release from adipose tissue into the blood decreases [10]. In the intestines, metformin inhibits glucose absorption and other simple carbohydrates. It increases the secretion of glucagon-like peptide-1 (GLP-1) by enterocytes, inhibits dipeptidyl-peptidase 4, and increases the activity of incretins [8].

Our results also demonstrate positive changes in lipid metabolism, both in the group of animals receiving metformin pellets and the tablet form of the drug. In particular, the blood content of total cholesterol and triglycerides in animals with the pelleted form of the drug decreased by 44.2 and 41.1 %, respectively. In hamsters administered metformin tablets, these indices decreased more (by 46.3 % and 44.0 % ($p<0.05$)). However, the difference did not reach statistically significant values.

Our results showed that the 30-day treatment of animals with experimental metabolic syndrome using metformin in bio-implants (pellets) is not inferior in efficacy to its use in traditional tablet form.

Even though metformin is generally considered a safe drug, the literature describes adverse reactions that may occur in some patients. The most common are gastrointestinal symptoms, such as nausea and vomiting, which usually appear in the early stages of taking the drug and are more common in women. Other side effects are abdominal pain or discomfort and diarrhea. There is evidence of a correlation between the duration and daily dose of metformin and the risk of vitamin B12 deficiency. Rare but severe side effects can also occur, including hypoglycemia, dehydration, and even acute hepatitis. One of the most severe side effects can be the risk of lactic acidosis. Metformin-induced lactic acidosis presents with myalgia, lethargy, dyspnea, abdominal pain, and confusion. Although infrequent (4.3 per 100,000 patient-years), it has a 40–50 % mortality rate. Predictors of its occurrence are liver dysfunction and concomitant diseases. Most often, lactic acidosis occurs due to an overdose of the drug. A systematic review of overdoses found that lower serum pH and higher serum lactate levels were associated with increased mortality [4].

Taking into account the above, at the next stage, it was essential to evaluate the general condition of animals against the background of drug administration, the stability of the obtained effects on carbohydrate metabolism (absence of episodes of hypoglycemia), as well as the lactate content in the blood of animals with metabolic syndrome treated with metformin, depending on the route of administration.

At the time of the formation of the metabolic syndrome, in addition to the above-mentioned biochemical changes in the blood serum and an increase in body weight, a deterioration in the general condition of the animals was noted. All the hamsters were frail and passive, and there was a significant deterioration of the fur condition (tousled, dull, and in places, there were foci of alopecia).

Monitoring the glucose level in experimental animals, carried out every 2 days of treatment, did not reveal fluctuations in glycemia and episodes of hypoglycemic states in animals of both experimental groups. It indicates the uniformity of metformin absorption when administered subcutaneously and the absence of overdose symptoms. We believe that using metformin in the form of implants permits the active substance to be released in stable microdoses, significantly reducing possible side effects and preventing significant hormonal fluctuations.

Against the background of using bio-implants in animals, the state of the fur improved significantly, in contrast to animals that received the tablet form of the drug and animals with metabolic syndrome without correction. The quality of fur (thickness and shine) was also better in animals with biopellets. In foci of alopecia, there was a moderate growth of covering hair, characterized by shine, and did not tend to fall out. There was a faster recovery of the fur in the foci of alopecia.

Four-week treatment of experimental animals with metformin led to increased lactate content. At the same time, in the group of animals prescribed metformin in tablets, this index increased by 64.9 %, while its increase in the group of animals that received metformin subcutaneously – by only 36.2 % ($p<0.05$).

## Conclusion

The results show that metformin in the form of bioidentical implants has an effective therapeutic effect in animals with experimental metabolic syndrome, reducing the signs of a pathological condition (corrects the level of glycemia, promotes normalization of body weight, reduces insulin resistance, and has a positive effect on lipid metabolism). According to these effects, it is not inferior to metformin, which animals receive intragastrically in tablets. At the same time, the metformin pellets didn't show episodes of hypoglycemia and, unlike the tablet form of administration, did not show an increased risk of lactic acidosis, and also had a positive effect on the general condition and appearance of experimental animals.

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