Stepanov G. F., Vastyanov R. S., Kostina A. A., Mokriienko E. M. Peculiarities of the relationship between the terminal site of glycolysis and the initial segment of gluconeogenesis in the myocardium and skeletal muscles of animals irradiated at different doses. Journal of Education, Health and Sport. 2023;47(1): 165-179. eISSN 2391-8306. DOI http://dx.doi.org/10.12775/JEHS.2023.47.01.016
https://apcz.umk.pl/JEHS/article/view/49266
https://zenodo.org/record/10804020

The journal has had 40 points in Ministry of Education and Science of Poland parametric evaluation. Annex to the announcement of the Minister of Education and Science of 17.07.2023 No. 32318. Has a Journal's Unique Identifier: 201159. Scientific disciplines assigned: Physical Culture Sciences (Field of Medical sciences and health sciences); Health Sciences (Field of Medical Sciences and Health Sciences). Punkty Ministerialne z 2019 - aktualny rok 40 punktów. Załącznik do komunikatu Ministra Edukacji i Nauki z dnia 17.07.2023 Lp. 32318. Posiada Unikatowy Identyfikator Czasopisma: 201159. Przypisane dyscypliny naukowe: Nauki o kulturze fizycznej (Dziedzina nauk medycznych i nauk o zdrowiu); Nauki o zdrowiu (Dziedzina nauk medycznych i nauk o zdrowiu).

Received: 29.07.2023. Revised:21.08.2023. Accepted: 28.08.2023. Published: 29.08.2023.

UDC: 614.876:616-055.6:577.122:616-092.4

PECULIARITIES OF THE RELATIONSHIP BETWEEN THE TERMINAL SITE OF GLYCOLYSIS AND THE INITIAL SEGMENT OF GLUCONEOGENESIS IN THE MYOCARDIUM AND SKELETAL MUSCLES OF ANIMALS IRRADIATED AT DIFFERENT DOSES

G. F. Stepanov, R. S. Vastyanov, A. A. Kostina, E. M. Mokriienko

Odesa National Medical University, Odesa, Ukraine

Abstract

Existing types of muscles differ not only structurally, but also metabolically (direction of energy exchange, choice of biosubstrates, dependence on the action of mediators, hormones, etc.). A number of studies have been devoted to the study of the impact of ionizing radiation on metabolism in muscle tissue. After irradiation, thickened muscle cells with enlarged nuclei appear in the heart muscle, the number of which does not decrease for 6 months, which indicates a violation of nuclear-cytoplasmic relations in myocardial cells. Phosphoenolpyruvate carboxykinase which ensures the utilization of cytoplasmic oxaloacetate and its transformation into phosphoenolpyruvate, completes the initial stage of gluconeogenesis and can limit the rate of gluconeogenesis from lactate. It should be emphasized that phosphoenolpyruvate carboxykinase is more active in skeletal muscles, where the activity of pyruvate kinase and lactate dehydrogenase is increased. The purpose of the work is to investigate the peculiarities of the relationship between the terminal site of glycolysis and the initial segment of

gluconeogenesis in the myocardium and skeletal muscles of animals irradiated at different doses. The authors proved that in animals irradiated at a dose of 0.5 Gy, the activity of pyruvate kinase in the myocardium and skeletal muscle increases compared to intact animals. In the blood, on the contrary, there is a decrease in the activity of this enzyme compared to intact animals. When animals are irradiated at a dose of 1.0 Gy, diametrically opposite changes are observed. When animals are irradiated at a dose of 0.5 Gy, there is a slight decrease in the activity of lactate dehydrogenase in the myocardium and blood against the background of an increase in the activity of this enzyme in skeletal muscle. Irradiation of animals at a dose of 1.0 Gy leads to an acute increase in lactate dehydrogenase activity in the myocardium and skeletal muscle. The data obtained revealed that in animals irradiated at a dose of 0.5 Gy, the activity of phosphoenolpyruvate carboxykinase in skeletal muscle increases, while in cardiac muscle, on the contrary, its activity decreases as well as in blood. With an increase in the radiation dose to 1.0 Gy, diametrically opposite changes in the activity of phosphoenolpyruvate carboxykinase are observed. The authors concluded that outlined changes in the way of intramuscular glycolysis and gluconeogenesis reactions show the pathophysiologcal mechanisms of biochemical supply restructuring as the result of ionizing irradiation influence. From the fundamental point of view these results show the direction of pathophysiologically oriented pharmacological correction of radiation-provoked muscle disturbances.

Key words: irradiated animals; skeletal muscle; myocardium; glcolysis; gluconeogenesis; pathophysiological mechanisms

Carbohydrates play a leading role in the bioenergetics of the body, and most of them enter the body with food, and a smaller part is resynthesized in the body from substances of non-carbohydrate origin (gluconeogenesis) [9].

The most important problem is the study of biocatalytic processes in organs and tissues, which allows us to assess the depth of pathochemical changes under various effects on the body. Existing types of muscle tissue (smooth muscles, myocardium, skeletal muscles) differ not only structurally, but also metabolically (direction of energy exchange, choice of biosubstrates, dependence on the action of mediators, hormones, etc.). Cardiac muscle differs from skeletal muscle not only in morphological and functional characteristics, but also primarily in the significant content of mitochondria, the speed of protein exchange, the high intensity of aerobic processes, in particular, the reactions of the tricarboxylic acid cycle, creatine phosphokinase. Cardiac muscle, unlike skeletal muscle, uses significant amounts of fatty acids, as well as lactate and ketone bodies, to obtain energy in addition to glucose [2].

A number of studies have been devoted to the study of the impact of ionizing radiation on metabolism in muscle tissue [3, 12]. It has been established that general irradiation of rats at doses of 0.5-4.5 Gy causes irreversible and dose-independent changes: edematous degeneration, intracellular lysis, damage to mitochondria of myocardial endotheliocytes, which can play a role in the development of deterministic, non-stochastic consequences of radiation in small doses. After irradiation, thickened muscle cells with enlarged nuclei appear in the heart muscle, the number of which does not decrease for 6 months, which indicates a violation of nuclear-cytoplasmic relations in myocardial cells. The morphometric characteristics of heart mitochondria also change, the contractile capacity of the myocardium is impaired. Blockades of His bundle branches, violations of the sinus node automatism are noted. In the myocardium of an irradiated organism, the efficiency of oxidative phosphorylation decreases not only in the cytochrome section of the respiratory chain of mitochondria, but also in the points of conjugation.

Phosphoenolpyruvate carboxykinase (PEPCK), which ensures the utilization of cytoplasmic oxaloacetate and its transformation into phosphoenolpyruvate, completes the initial stage of gluconeogenesis and can limit the rate of gluconeogenesis from lactate. A characteristic feature is that the activity of PEPCK in muscles is more than 3 times higher than that in the heart. It should be emphasized that PEPCK is more active in skeletal muscles, where the activity of pyruvate kinase and lactate dehydrogenase is increased. It can be concluded that the studied enzyme shows greater activity in tissue characterized by a high intensity of glycolysis and a low capacity for aerobic oxidation. To confirm this, it is worth mentioning that the liver, which has the greatest activity of PEPCK and gluconeogenesis in general, has a great ability to oxidize carbohydrates through the glycolytic pathway [4].

The aim of the work is to investigate the peculiarities of the relationship between the terminal site of glycolysis and the initial segment of gluconeogenesis in the myocardium and skeletal muscles of animals irradiated at different doses.

Material and Methods

The studies were conducted on sexually mature male rats weighing 180- 220 g. of Wistar line kept on a standard vivarium diet. Keeping, processing of the animals and manipulations with them were carried out in accordance with the "General Ethical Principles of Animal Experiments" adopted by the Fifth National Congress on Bioethics (Kyiv, 2013), guided by the recommendations of the European Convention on the Protection of Vertebrate Animals for Experimental and Other Scientific Purposes (Strasbourg, 1985), the methodological recommendations of the State Expert Center of the Ministry of Health

Ukraine "Preclinical studies of drugs" (2001) and the rules of humane treatment of experimental animals and conditions approved by the Bioethics Commission of the Odesa National Medical University (protocol No. 32D dated 03/17/2016).

The animals were divided into groups as follows:

1. Intact sexually mature animals.
2. Mature animals irradiated at a dose of 0.5 Gy.
3. Mature animals irradiated at a dose of 1.0 Gy.

There were 7-10 animals in each group.

Animals were removed from the experiment by euthanasia under propofol (IV, 60 mg/kg) anesthesia. After the animals were dissected, blood was collected, the heart and the anterior group of thigh muscles were removed. The removed cardiac and skeletal muscles were washed with chilled 0.9% physiological NaCl solution, minced and homogenized in a 9-fold volume of 0.32 mol sucrose at 0.05 mol Tris buffer, pH 7.36 in a homogenizer with Teflon surfaces and subjected to differential centrifugation in a refrigerated centrifuge RC-6. Nuclei were precipitated at 1000g for 10 min., then mitochondria at 12000g for 20 min., resuspended in a homogenizer in isolation medium containing 0.1% triton X-100 solution at the rate of 1 ml of 0.1% triton solution per 500 mg of tissue and left in ice for 30-35 min.

To determine biochemical indicators in tissues, they were subjected to differential centrifugation [5]. To detect the content of biosubstrates in tissues, they were immersed in liquid nitrogen, deproteinized with 0.6 N perchloric acid. The protein precipitate was separated by centrifugation for 15 min. at 3000 g . The total amount of protein in the muscles was determined by the spectrophotometric biuret method [6]. We focused our attention on the determination of activity of enzymes and substrates of the terminal site of glycolysis and the initial segment of gluconeogenesis in the myocardium and skeletal muscles of animals irradiated at different doses.

The principle of the method for determining the activity of pyruvate kinase is to convert phosphoenolpyruvate into pyruvate in the presence of ADP, which in turn, in the presence of reduced NAD and lactate dehydrogenase, is converted into lactate, thereby oxidizing $NADH+H^+$ [14]. Pyruvate kinase activity was expressed in nmol of pyruvate per mg of protein in the sample in 1 min of incubation.

The principle of the method for determining the activity of lactate dehydrogenase consists in the reduction of pyruvate to lactate in the presence of reduced NAD [7]. LDH activity was expressed in μmoles of the used $NADH+H^+$ per mg of protein in the sample in 1 min of incubation.

LDH isoenzymes in tissues and blood were detected using electrophoresis in a polyacrylamide gel at a temperature of +30 $^{C.}$ Electrophoregrams were stained with a substrate mixture (NAD, tetrazolium nitroblue, sodium lactate, phenazine metasulfate, phosphate buffer). After fixing the electrophoregrams, they were dried in a thermostat for 2 hours at a temperature of +50 ^{0} C and densitometered. The content of isoenzymes was determined planimetrically [8].

The principle of the method for determining the activity of phosphoenolpyruvate carboxykinase is to convert phosphoenolpyruvate into oxaloacetate in the presence of inosine diphosphate, which in the presence of reduced NAD and malate dehydrogenase is converted into malate, thereby oxidizing NADH [15]. Enzyme activity was expressed in nmol of oxidized NADH per mg of protein in the sample in 1 min of incubation.

The principle of the method for detecting the content of lactate and pyruvate consists in an enzymatic reaction catalyzed by LDH in the presence of the oxidized or reduced form of NAD, the accumulation or loss of which is recorded spectrophotometrically at 340 nm against the control, where there is no tissue extract, and expressed in μmol per 1g of tissue [7]. The protein content in the samples was determined by the biuret method [7].

The obtained data were subjected to statistical processing by the method of estimating the average with the help of "T-tables" using the χ^2 criterion and computer programs. The minimum statistical probability was determined at $p<0.05$.

Results

Studying the peculiarities of the relationship between the terminal site of glycolysis and the initial segment of gluconeogenesis in the myocardium and skeletal muscles of animals irradiated at different doses, it was established that in animals irradiated at a dose of 0.5 Gy, the activity of pyruvate kinase in the myocardium and skeletal muscle increases compared to intact animals. In the blood, on the contrary, there is a decrease in the activity of this enzyme compared to intact animals. When animals are irradiated at a dose of 1.0 Gy, diametrically opposite changes are observed, a decrease in the activity of the studied enzyme in skeletal muscle and an increase in activity in cardiac muscle, while a slight increase in its activity is observed in blood (Table 1).

Thus, with an increase in the radiation dose, there is a decrease in the processes of substrate phosphorylation in skeletal muscle and their intensification in the myocardium, and an increase in the activity of this enzyme in the blood may indicate a decrease in the ability of muscle tissue to fix this enzyme in the cell due to an increase in the permeability of plasma membranes.

Table 1.

Activity of pyruvate kinase in myocardium, skeletal muscle and blood serum of intact and irradiated animals (n=10)

Groups of animals	Activity of pyruvate kinase (M±m), nmol/mg of protein in 1 min		
	Myocardium	Skeletal muscle	Blood serum
Intact	96.8±5.4	282.3±15.3	10.25±0.90
Irradiation at 0.5 Gy	98.2±5.2	308.4±16.8	8.76±0.82
Irradiation at 1.0 Gy	112.6 ± 6.4	264.8 ± 15.2	10.94 ± 0.92

Experimental studies were conducted aimed at studying the activity of lactate dehydrogenase, which is detected by the reduction of pyruvate to lactate (the terminal reaction of anaerobic glycolysis) in the cytoplasm of cardiac and skeletal muscles, as well as in the blood serum of animals exposed to radiation at a dose of 0.5 Gy and 1,0 Gy (Table 2).

Table 2.

Lactate dehydrogenase activity in the myocardium, skeletal muscle, and blood serum of intact and irradiated animals (n = 10)

Groups of animals	Lactate dehydrogenase activity (M ±m)		
	Myocardium	Skeletal muscle	Blood serum
Intact	1.542±0.076	2.060±0.094	8.118±0.545
Irradiation at 0.5 Gy	1.025±0.022 *	2.434±0.096	7.956±0.524
Irradiation at 1.0 Gy	3.82±0.39 *	4.18±0.36 *	10.82±0.51 *

Notes:

1. The activity of lactate dehydrogenase in the myocardium and skeletal muscles is expressed in μmol/mg of protein in 1 min.; in blood serum - in nmol/mg of protein in 1 minute;

2. * - $P<0.05$ – probable differences of the studied indicators compared to the corresponding indicators in intact animals.

When animals are irradiated at a dose of 0.5 Gy, there is a slight decrease in the activity of lactate dehydrogenase in the myocardium and blood against the background of an increase in the activity of this enzyme in skeletal muscle.

Irradiation of animals at a dose of 1.0 Gy leads to an acute increase in LDH activity in the myocardium and skeletal muscle. In the cytoplasm of the cardiac muscle of irradiated animals, the activity of LDH is 2.43 times higher than that in the myocardium of intact rats, and in the cytoplasm of skeletal muscle - 2 times, and these changes are reliable. The activity of the enzyme in blood serum is 1.6 times higher than the activity of the enzyme in intact animals, which is a consequence of the development of anaerobic processes in both types of muscle tissue and impaired permeability of plasma membranes during irradiation.

The obtained data on the activity of lactate dehydrogenase (LDH) correlate with the indicators of its isoenzyme spectrum (Table 3).

Table 3.

Isoenzyme spectrum of lactate dehydrogenase of myocardium and skeletal muscle of sexually mature animals irradiated at different doses (n = 10)

Substances under study	Activity of lactate dehydrogenase and its isoforms (M ±m)			
	Myocardium		Skeletal muscle	
	Irradiated at a dose of 0.5 Gy	Irradiated at a dose of 1.0 Gy	Irradiated at a dose of 0.5 Gy	Irradiated at a dose of 1.0 Gy
LDH $_1$ %	36.4 ± 0.9	34.4 ± 0.6	0.8 ± 0.2	0.6 ± 0.2
LDH $_2$ %	35.3 ± 0.9	33.8 ± 0.8	2.4 ± 0.2	1.6 ± 0.2*
LDH $_3$ %	24.8 ± 0.7	24.2 ± 0.4	9.8 ± 0.6	9.8 ± 0.6
LDH $_4$ %	4.2 ± 0.4	5.8 ± 0.8	14.1 ± 1.2	19.6 ± 1.3*
LDH $_5$ %	0.4 ± 0.1	1.4 ± 0.2	75.4 ± 2.1	128.4 ± 2.8*

Note. * - $P<0.05$ – probable differences of the studied indicators compared to the corresponding indicators in intact animals.

The LDH isoenzyme spectrum of the myocardium in animals irradiated at a dose of 0.5 Gy is characterized by a slight increase in the rapidly migrating LDH $_1$ and LDH_2 isoenzymes to the anode, and a decrease in the amount of LDH $_4$ and, especially, LDH_5. With an increase in the radiation dose to 1.0 Gy, opposite changes are observed in the LDH isozyme spectrum, i.e. a decrease in the activity of the isoenzymes LDH_1 and LDH_2 , which quickly migrate to the anode, against the background of an increase in the activity of LDH_4 and, especially, LDH_5, whose activity is twice as high as this indicator in intact animals.

The LDH isoenzyme spectrum of skeletal muscles in animals irradiated at a dose of 0.5 Gy is characterized by a slight decrease in the fast-migrating LDH_1 and LDH_2 isozymes to

the anode, and a gradual increase in the slowly migrating LDH_4 and, especially, LDH_5 to the anode. Deeper changes in the isozyme spectrum of LDH are observed in the skeletal muscles of animals irradiated at a dose of 1.0 Gy, where, against the background of a sharp decrease in the content of the isoenzymes LDH_1 and LDH_2 , which migrate rapidly to the anode , by almost two times, there is a twofold increase in LDH_4 and LDH_5 migrating slowly to the anode.

Phosphoenolpyruvate carboxykinase (PEPCK) is characterized by multidirectional changes in its activity in skeletal and cardiac muscles (Table 4).

Table 4.

Activity of phosphoenolpyruvate carboxykinase in the myocardium, skeletal muscle and blood serum of intact and irradiated animals (n=10)

Groups of animals	Activity of phosphoenolpyruvate carboxykinase (M ±m)		
	Myocardium	Skeletal muscle	Blood serum
Intact	17.726 ± 1.151	56.544 ± 1.978	0.933 ± 0.096
Irradiation at 0.5 Gy	16.128 ± 1.124	57.652 ± 1.986	0.886 ± 0.082
Irradiation at 1.0 Gy	18.216 ± 1.162	55.924 ± 1.926	0.989 ± 0.098

Note. The activity of phosphoenolpyruvate carboxykinase is expressed in nmol/mg of protein in 1 min.

Thus, in animals irradiated at a dose of 0.5 Gy, the activity of PEPCK in skeletal muscle increases, while in heart muscle, on the contrary, its activity decreases, as well as in blood. With an increase in the radiation dose up to 1.0 Gy, diametrically opposite changes in the activity of PEPCK are observed, where the activity of this enzyme in cardiac muscle increases compared to intact animals, and in skeletal muscle, on the contrary, its activity decreases, as in blood.

The obtained results regarding the activity of enzymes of glycolysis, gluconeogenesis, substrate phosphorylation and isoenzymes of lactate dehydrogenase are correlated with the content of their metabolites - pyruvate and lactate. Thus, the content of lactate in skeletal muscle increases with an increase in the radiation dose and, in animals irradiated at a dose of 1.0 Gy, exceeds this indicator almost twice (Table 5), in contrast to the concentration of pyruvate, where, in animals irradiated at a dose of 0.5 Gy, this indicator increases slightly, and in animals irradiated at a dose of 1.0 Gy, this indicator is almost 2 times lower compared to intact animals (Table 6).

Table 5.

Lactate content in the myocardium, skeletal muscle, and blood serum of intact and irradiated animals (n=10)

Groups of animals	Lactate content (M±m)		
	Myocardium	Skeletal muscle	Blood serum
Intact	2.768 ± 0.191	3.327 ± 0.165	1.067 ± 0.072
Irradiation at 0.5 Gy	1.986 ± 0.168*	3.786 ± 0.172	1.024 ± 0.056
Irradiation at1.0 Gy	3.162 ± 0.196	6.544 ± 0.236*	1.426 ± 0.082*

Notes:

1. Lactate content is expressed in μmol/g of tissue, in blood - in μmol/ml;

2. * - P<0.05 – probable differences of the studied indicators compared to the corresponding indicators in intact animals.

Table 6.

Pyruvate content in the myocardium, skeletal muscle, and blood serum of intact and irradiated animals (n=10)

Groups of animals	Pyruvate content (M±m)		
	Myocardium	Skeletal muscle	Blood serum
Intact	0.310 ± 0.015	0.332 ± 0.018	0.130 ± 0.006
Irradiation at 0.5 Gy	0.524 ± 0.028*	0.358 ± 0.016	0.102 ± 0.004*
Irradiation at 1.0 Gy	0.292 ± 0.014	0.178 ± 0.012*	0.158 ± 0.006*

Notes:

1. Pyruvate content is expressed in μmol/g of tissue, in blood - in μmol/ml;

2. * - P<0.05 – probable differences of the studied indicators compared to the corresponding indicators in intact animals.

In the myocardium of animals irradiated at a dose of 0.5 Gy, the content of lactate slightly decreases against the background of an unreliable increase in the content of pyruvate, while with an increase in the dose of irradiation up to 1.0 Gy, the content of these metabolites is diametrically opposite, namely, against the background of a slight decrease in pyruvate in the myocardium, lactate content increases.

Unidirectional changes in pyruvate and lactate content are observed in the blood of animals irradiated at different doses. Thus, in the blood of animals irradiated at a dose of 0.5

Gy, a decrease in the content of lactate and pyruvate is observed, while in the blood of animals irradiated at a dose of 1.0 Gy, an increase of these indicators is observed compared to intact animals.

Thus, evaluating the obtained results, it should be noted that irradiation of sexually mature animals causes an increase in the activity of lactate dehydrogenase in the blood serum, which reflects the strengthening of glycolytic processes in the muscle tissue in pathology and correlates with the activation of direct malate dehydrogenase in the blood serum, which indicates the development of acidosis and violation of the permeability of the membranes of muscle cells .

Discussion

A significant amount of energy required for muscle contraction is obtained as a result of aerobic or anaerobic oxidation of carbohydrates, and the predominant role of each of them depends on the type of muscle tissue and the conditions in which the body is [13, 16]. Of particular interest is the place of glycolytic substrate phosphorylation in the energy supply of the myocardium and skeletal muscles, the relationship between the terminal site of glycolysis and the final stage of the tricarboxylic acid cycle, the role of their metabolites and the competition of enzymes for cytoplasmic $NADH^{+}H^{+}$ and in the transport of reduced equivalents from the sarcoplasm to the mitochondria.

Only about 10% of the lactate formed in the muscles is excreted by blood, and the removal of lactate from the muscles occurs mainly due to the resynthesis of glycogen from lactate. Since the pyruvate kinase reaction is irreversible, the participation of lactate and pyruvate in the resynthesis of carbohydrates is carried out through a number of additional reactions, and in muscle tissue such reactions can be NADPH-dependent malate dehydrogenase, which catalyzes the interconversion of pyruvate into malate, and phosphoenolpyruvate carboxykinase, which provides the synthesis of the initial product of gluconeogenesis phosphoenolpyruvate from oxaloacetate [9].

Studying the ways of utilization and resynthesis of phosphoenolpyruvate - an important component of glycolytic substrate phosphorylation, a precursor of pyruvate and lactate in tissues, and, at the same time, one of the starting products of gluconeogenesis, draws attention to the difference in the state of biochemical processes that ensure this stage of metabolism. Skeletal muscle, as already indicated, is characterized by high activity of glycolytic processes, and this is reflected in the activity of enzymes that catalyze glycolysis reactions and in the content of metabolites.

Glycolysis and gluconeogenesis are closely interdependent, because the reaction products of one process are substrates for the other and the regulation of the connection between glycolysis and gluconeogenesis can be carried out by reversibly switching the flow of intermediate products from one pathway to another [11]. However, it has not been clarified what the specifics of the relationship between the terminal site of glycolysis and the initial segment of gluconeogenesis in the myocardium and skeletal muscles of animals irradiated at different doses are, since cataplerotic and anaplerotic reactions in muscle tissue affect the energy exchange of the entire organism.

Studying the peculiarities of the relationship between the terminal site of glycolysis and the initial segment of gluconeogenesis in the myocardium and skeletal muscles of animals irradiated at different doses, it was established that in animals irradiated at a dose of 0.5 Gy, the activity of pyruvate kinase in the myocardium and skeletal muscle increases compared to intact animals. In the blood, on the contrary, there is a decrease in the activity of this enzyme compared to intact animals. When animals are irradiated at a dose of 1.0 Gy, diametrically opposite changes are observed, a decrease in the activity of the studied enzyme in skeletal muscle and an increase in activity in cardiac muscle, while a slight increase in its activity is observed in blood.

When animals are irradiated at a dose of 0.5 Gy, there is a slight decrease in the activity of lactate dehydrogenase in the myocardium and blood against the background of an increase in the activity of this enzyme in skeletal muscle. Irradiation of animals at a dose of 1.0 Gy leads to a sharp increase in LDH activity in the myocardium and skeletal muscle. In the cytoplasm of cardiac muscle of irradiated animals, LDH activity is 2.43 times higher than in the myocardium of intact rats, and in the cytoplasm of skeletal muscle, it is 2 times higher. The activity of the enzyme in blood serum is 1.6 times higher than the activity of the enzyme in intact animals ($p < 0.05$), which is a consequence of the development of anaerobic processes in both types of muscle tissue and impaired permeability of plasma membranes during irradiation.

In animals irradiated at a dose of 0.5 Gy, the activity of PEPCK in skeletal muscle increases, while in cardiac muscle, on the contrary, its activity decreases as well as in blood. With an increase in the radiation dose to 1.0 Gy, diametrically opposite changes in the activity of PEPCK are observed, where the activity of this enzyme in cardiac muscle increases compared to intact animals, and in skeletal muscle, on the contrary, its activity decreases, as in blood.

Our results regarding the activity of enzymes of glycolysis, gluconeogenesis, substrate phosphorylation and LDH isoenzymes are correlated with the content of their metabolites -

pyruvate and lactate. Thus, the content of lactate in skeletal muscle increases with an increase in the radiation dose and, in animals irradiated at a dose of 1.0 Gy, exceeds this indicator by almost two times, in contrast to the concentration of pyruvate, where, in animals irradiated at a dose of 0.5 Gy, this indicator increases slightly, and in animals irradiated at a dose of 1.0 Gy this indicator is almost 2 times lower compared to intact animals.

Resuming, one should conclude that there are few similar scientific works devoted strogly to ionizing iradiaton impact on glycolysis and gluconeogenesis manifestation and on internal organisms' metabolism in general, mainly of dmestic authors [1]. We read many scientific observations concerning cancer cases treatment and therefore those data were sometimes close to ours [10]. The main idea of our investigations is to demonstrate the way and mechanisms of biological organism adapatation to the harmful influence of ionizing irradiation. Our data concerning changes in the way of intramuscular glycolysis and gluconeogenesis reactions show the pathophysiologcal mechanisms of biochemical supply restructuring as the result of ionizing irradiation influence. From the fundamental point of view these results show the direction of pathophysiologically oriented pharmacological correction of radiation-provoked muscle disturbances.

Conclusions

The obtained data highlighted interesting points regarding the relationship between the terminal site of glycolysis and the initial segment of gluconeogenesis in the myocardium and skeletal muscles of animals irradiated at different doses.

Studying the peculiarities of the relationship between the terminal site of glycolysis and the initial segment of gluconeogenesis in the myocardium and skeletal muscles of animals irradiated at different doses, it was established that in animals irradiated at a dose of 0.5 Gy, the activity of pyruvate kinase in the myocardium and skeletal muscle increases compared to intact animals. In the blood, on the contrary, there is a decrease in the activity of this enzyme compared to intact animals. When animals are irradiated at a dose of 1.0 Gy, diametrically opposite changes are observed, a decrease in the activity of the studied enzyme in skeletal muscle and an increase in activity in cardiac muscle, while a slight increase in its activity is observed in blood.

Thus, with an increase in the radiation dose, there is a decrease in the processes of substrate phosphorylation in skeletal muscle and their intensification in the myocardium, and an increase in the activity of this enzyme in the blood may indicate a decrease in the ability of muscle tissue to fix this enzyme in the cell due to an increase in the permeability of plasma membranes.

When animals are irradiated at a dose of 0.5 Gy, there is a slight decrease in the activity of LDH in the myocardium and blood against the background of an increase in the activity of this enzyme in skeletal muscle. Irradiation of animals at a dose of 1.0 Gy leads to a sharp increase in LDH activity in the myocardium and skeletal muscle. In the cytoplasm of cardiac and skeletal muscle of irradiated animals, LDH activity exceeds that of intact rats. The activity of the enzyme in blood serum exceeds the activity of the enzyme in intact animals by several times, which is a consequence of the development of anaerobic processes in both types of muscle tissue and disruption of the permeability of plasma membranes during irradiation.

In animals irradiated at a dose of 0.5 Gy, the activity of PEPCK in skeletal muscle increases, while in cardiac muscle, on the contrary, its activity decreases as well as in blood. With an increase in the radiation dose to 1.0 Gy, diametrically opposite changes in the activity of PEPCK are observed, where the activity of this enzyme in cardiac muscle increases compared to intact animals, and in skeletal muscle, on the contrary, its activity decreases, as in blood.

Our results regarding the activity of enzymes of glycolysis, gluconeogenesis, substrate phosphorylation and lactate dehydrogenase isoenzymes are correlated with the content of their metabolites - pyruvate and lactate. Thus, the content of lactate in skeletal muscle increases with an increase in the dose of irradiation, in contrast to the concentration of pyruvate, where, in animals irradiated at a dose of 0.5 Gy, this indicator increases slightly, and in animals irradiated at a dose of 1.0 Gy, this indicator is almost 2 times lower compared to intact animals.

Our data concerning changes in the way of intramuscular glycolysis and gluconeogenesis reactions show the pathophysiologcal mechanisms of biochemical supply restructuring as the result of ionizing irradiation influence. From the fundamental point of view these results show the direction of pathophysiologically oriented pharmacological correction of radiation-provoked muscle disturbances.

References

1. Andrushkiv B. Neglecting the problems of the Chernobyl tragedy is a consequential chain of the emergence of modern problems of a full-scale war with Russia. Science and information bulletin of the National Academy of Sciences of Higher Education of Ukraine. 2022; 1(112): 13-18 [In Ukrainian].

2. Biochemical foundations of physical culture and sports: Study guide for students of higher educational institutions of physical culture and sports. Uzhhorod : Publishing House JV “PoliPrint”. 2014. 91 [In Ukrainian].

3. Gubsky YuI, Nizhenkovska IV, Korda MM. Biological and Bioorganic Chemistry. Book 2. Biological Chemistry. 2021. 544 [In Ukrainian].

4. Cherkasova LS, Myronova TM. Effect of ionizing radiation on carbohydrate metabolism enzymes. Radiobiology. 1976; 16(5): 657–664 [In Russian].

5. Kochetov HA. A practical guide to enzymology. Moscow : High School. 1971. 352 [In Russian].

6. Lapovets LE, Lebed GB, Yastremska OO. Clinical laboratory diagnostics. Kyiv: Medicine. 2019. 472 [In Ukrainian].

7. Nakonechna OA, Bachynskyi RO. Biochemistry of enzymes. Aspects of medical enzymology. Kharkiv. 220. 48 [in Ukrainian].

8. Mardashko AA, Popik GS. Method for obtaining electrophoregrams of protein substances. Patent No 1196771. Bulletin of inventions and discoveries. 1985; 45: 174 [In Russian].

9. Baynes J., Dominiczak M. Medical Biochemistry. 5th Edition. Elsevier, 2018. 712 p.

10. Dabos KJ, Parkinson JA, Sadler IH, Plevris JN, Hayes PC (1)H nuclear magnetic resonance spectroscopy-based metabonomic study in patients with cirrhosis and hepatic encephalopathy. World J Hepatol. 2015; 7(12): 1701-1707. doi: 10.4254/wjh.v7.i12.1701.

11. Hernández F Glycolysis and gluconeogenesis: A teaching view. J Biol Chem. 2021; 296:100016. doi: 10.1016/j.jbc.2020.100016.

12. Lippincott Illustrated Reviews: Biochemistry. Philadelphia : Wolters Kluwer, 2017. 560 p.

13. Moroz VM, Shandra OA, Vastyanov RS, Yoltukhivsky MV, Omelchenko OD. Physiology. Vinnytsia : Nova Knyha, 2016: 722.

14. Muñoz ME, Ponce E. Pyruvate kinase: current status of regulatory and functional properties. Comparative biochemistry and physiology Part B: biochemistry and molecular biology. 2003; 135(2): 197-218. doi: https://.org/10.1016/S1096-4959(03)00081-2.

15. Opie LH, Newsholme EA. The activities of fructose 1,6 -diphosphatase, phosphofructokinase and phosphoenolpyruvate carboxykinase in white muscle and red muscle. Biochem J. 1967; 103(2): 391-399. doi : 10.1042/bj1030391.

16. Stepanov G.F., Vastyanov R.S. The peculiarities of low-dose ionizing radiation influence on muscles metabolism in experimental animals. World of Medicine and Biology. 2023; 2(84): 233-238. doi: 10.26724/2079-8334-2023-2-84-233-238

Conceptualization, (Stepanov G.F. & Vastyanov R.S.); methodology, (Stepanov G.F. & Kostina A.A.); formal analysis, (Kostina A.A.); data curation, (Mokriienko E.M.); writing—original draft preparation, (Mokriienko E.M.); writing—review and editing, (Kostina A.A.); supervision (Stepanov G.F. & Vastyanov R.S.).
All authors have read and agreed to the published version of the manuscript.

Funding
This research received no external funding.

Institutional Review Board Statement
The experimental studies were carried out in the conditions of a chronic experiment in accordance with international standards of humane treatment of vertebrate animals and approved by the Ethics Committee of Odesa National Medical University (N7/21, 11 October 2021)

Informed Consent Statement
The data of experimental studies are given. Written informed consent from the patients was not necessary to publish this paper.

Data Availability Statement
The data presented in this study are available on request from the corresponding author.

Conflicts of Interest
The authors declare no conflict of interest.