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Therapeutic Potential of Zinc ^{64}Zn Aspartate for Obesity Management: Impact on Oxidative Stress, Lipid Metabolism, Pancreas and Liver in High-Calorie Diet Model

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Summary

Zinc is a critical micronutrient that plays multifaceted roles in oxidative stress management, lipid metabolism, pancreatic function, and liver health, which are all closely interconnected with obesity. Maintaining adequate zinc levels is essential for overall metabolic health and proper functioning of these vital systems.

The investigational new drug complex of zinc-64 aspartate (KLS-1 or ^{64}Zn-aspartate) was evaluated in this study as a pharmaceutical agent targeting oxidative stress and lipid metabolism using rodent model of obesity. KLS-1 is the isotopically modified zinc aspartate in which stable (non-radioactive) ^{64}Zn atoms were enriched to exceed 99% atomic fraction of total zinc, as compared to natural isotopic ratio of 64Zn of 48.6%. In this paper, we discuss our findings and the effects rendered by KLS-1 on lipid metabolism, pancreas and liver function.

This study was conducted on outbred rats, which were divided into 4 experimental groups: 1) control group consuming standard food (3.81 kcal/g), 2) obese group consuming a high-calorie diet (5.35 kcal/g), 3) obese group consuming a high-calorie diet (5.35 kcal/g) treated with intragastric administration of ^{64}Zn- aspartate at a dose of 4.5 mg per animal during 6 weeks (the ***obese rats***), 4) the group consuming standard food diet (3.81 kcal/g) with ^{64}Zn- aspartate form administration. The obese rats treated with ^{64}Zn-64 stable isotope demonstrated decreased area of the hepatocytes, insulin and glucose levels in serum; increased catalase and superoxide dismutase activity, and area of pancreatic islets in comparison with the obese group.

The study shows that ^{64}Zn-aspartate is effective as a therapeutic agent for obesity management, significantly reducing body mass, improving histopathological changes in the pancreas and liver and normalizing oxidative stress in high-calorie diet animal models. These findings suggest that ^{64}Zn-

38 aspartate may be a promising monotherapy or adjunct treatment for obesity, offering benefits in
39 weight reduction, organ protection, and antioxidant balance.

40 1 Introduction

41 Zinc is a critical trace element essential for human health throughout the entire lifespan, from
42 fetal development to old age. It is classified among the indispensable trace elements and essential
43 micronutrients, alongside iron, iodine, copper, selenium, and manganese, among others [1].
44 Zinc plays a fundamental role in maintaining the metabolic equilibrium of the human body. It is
45 involved in the function of over 200 enzymes, either as a structural component or as a regulator of
46 their activity, spanning all enzyme classes [2]. These include transferases (e.g., RNA and DNA
47 polymerases, reverse transcriptase, thymidine kinase, nucleotidyl transferase, carboxypeptidase, and
48 other peptidases), hydrolases (e.g., alkaline phosphatase, 5-nucleotidase, aminopeptidase), lyases
49 (e.g., aldolase, carbonic anhydrase), oxidoreductases (e.g., alcohol dehydrogenase, superoxide
50 dismutase), as well as ligases and isomerases [3, 4, 5, 6, 7, 8]. The absence of zinc renders the
51 metabolism of proteins, fats, and carbohydrates impossible.
52 The metabolic and structural importance of zinc is underscored by its extensive biological
53 activity. Zinc is crucial for processes such as cell division and differentiation (including growth,
54 tissue regeneration, and spermatogenesis) [9]. It plays an active role in nucleic acid metabolism and
55 protein synthesis [10]. Furthermore, zinc is vital for the metabolism of polyunsaturated fatty acids
56 and the conversion of prostaglandins [11]. It exhibits significant lipotropic activity and possesses
57 hepatoprotective properties [12, 13]. Zinc deficiency can negatively impact erythropoiesis and
58 hemoglobin synthesis [14].
59 Zinc plays a critical role in the metabolism and function of various endocrine glands, including
60 the pituitary, adrenal glands, pancreas, prostate, and testes [15]. It is present within the cells of the
61 anterior pituitary gland, where it participates in metabolic processes and modulates the activity of
62 hypophysiotropic hormones [16]. Zinc is instrumental in appetite control and glucose metabolism,
63 which may contribute to its potential benefits in managing obesity [17]. Zinc also extends and
64 enhances the action of adrenocorticotropic hormone (ACTH), amplifies the effects of gonadotropins
65 and growth hormone, and is crucial in the synthesis and biological activity of insulin [18].
66 Additionally, zinc acts as both a synergist and antagonist in the absorption and metabolism of various
67 trace elements and vitamins, such as iron, copper, magnesium, and vitamins A, E, and folic acid [19].
68 Zinc is thus integral to numerous essential physiological processes in the human body. Despite its
69 importance, the full extent of zinc's functions remains incompletely understood, with many
70 mechanisms of its action still under investigation. However, existing experimental and clinical
71 studies suggest that zinc is a key element in the body, and its deficiency is linked to the onset and
72 progression of several prevalent non-communicable diseases [20]. Zinc deficiency has been
73 described as "the most prevalent malnutrition in the world" [21]. The situation is even more
74 concerning in developing nations. According to World Health Organization (WHO) estimates,
75 approximately 31% of the global population is affected by zinc deficiency, with prevalence rates
76 ranging from 4% to 73% depending on the country. Multiple studies have demonstrated that blood
77 zinc levels were significantly decreased in obese patients [22]. A meta-analysis of 23 observational
78 studies found that individuals with obesity had lower concentrations of serum zinc than individuals
79 without obesity [23]. Zinc deficiency weakens various cellular functions required for zinc
80 metabolism, including disrupting zinc transport systems, impairing energy metabolism, affecting
81 protein synthesis and function, fading antioxidant defenses, and compromising immune function.
82 These effects create a self-exacerbating cycle where zinc deficiency further impairs the cellular
83 mechanisms needed for proper zinc utilization and homeostasis.

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84 Zinc appears to have antioxidant and anti-inflammatory properties that may help counteract the
85 oxidative stress and chronic inflammation associated with obesity [24, 25], which can lead to a
86 decrease in fat accumulation and improve overall metabolic health. Additionally, zinc administration
87 has been shown to mitigate the adverse effects of obesity on adipose tissue function, further
88 supporting its potential therapeutic role in obesity and its comorbidities as diabetes mellitus,
89 metabolic syndrome, etc. [26, 27]. In patients with metabolic syndrome, zinc supplementation
90 improved glycemic control, lipid profiles, and reduced inflammatory marker [28]. This indicates zinc
91 may have therapeutic potential for addressing multiple aspects of metabolic dysfunction in obesity. A
92 key laboratory indicator of zinc deficiency is a reduction in its concentration within blood plasma
93 (serum). However, this marker is highly variable, being influenced by numerous factors such as
94 dietary habits, circadian rhythms, age, sex, pregnancy, use of hormonal contraceptives, medications,
95 infections, oncological conditions, inflammatory processes, injuries, and excessive dietary iron intake
96 [29].
97 Zinc deficiency is considered a risk factor for several metabolic diseases, including non-alcoholic
98 fatty liver disease (NAFLD), obesity, and type 2 diabetes. Zinc deficiency can develop due to various
99 causes, including thyroid gland dysfunction, liver disease, impaired zinc absorption in the
100 gastrointestinal tract, and insufficient zinc levels in water and food sources [30]. The need for zinc
101 increases during pregnancy, significant physical exertion, stress, hemodialysis, and consumption of
102 foods high in phytic acid, which binds zinc and hinders its absorption. The risk of zinc deficiency is
103 further heightened by the prolonged use of certain medications (e.g., estrogens, corticosteroids,
104 diuretics, etc.) and excessive alcohol consumption [31].
105 Given that many essential metabolic processes are dependent on zinc-containing and zinc-
106 dependent enzymes, zinc deficiency can disrupt numerous physiological functions including
107 disrupting zinc transport systems, impairing energy metabolism, affecting protein synthesis and
108 function, fading antioxidant defenses, and compromising immune function. These effects create a
109 self-exacerbating cycle where zinc deficiency further impairs zinc metabolism and cellular
110 mechanisms needed for proper zinc utilization and homeostasis.
111 Extensive research has demonstrated a link between zinc deficiency and the development of
112 obesity, insulin resistance, type 2 diabetes, atherosclerosis, hypertension, and coronary heart disease
113 [32]. Zinc deficiency not only contributes to various metabolic disorders, which, if left uncorrected,
114 may trigger the onset of pathological conditions in individuals with genetic predispositions or
115 existing metabolic disturbances, but it also exacerbates the pathogenesis of certain systemic diseases.
116 In such cases, zinc deficiency acts as an additional pathogenetic factor, promoting disease
117 progression and increasing the likelihood of associated complications.
118 The increasing prevalence of obesity, its association with a multitude of other diseases and risk
119 factors that lead to disability and elevated mortality rates, along with the substantial financial burden
120 of providing medical care to individuals with obesity, position obesity as a significant medical,
121 social, and economic challenge in modern society. Epidemiological studies have highlighted the
122 rising prevalence of obesity across all economically developed countries [33].
123 The increasing prevalence of obesity across various population segments—regardless of social
124 and professional status, place of residence, age, or gender—elevates obesity to the level of a socially
125 significant issue, necessitating immediate action and innovative approaches to its prevention and
126 treatment [34].
127 Obesity has many causes, so its treatment should focus on using medications that help restore the
128 body's normal functions instead of just blocking or reducing them. However, the drugs currently
129 available for treating obesity can cause serious side effects and typically result in only an eight to ten
130 percent reduction in body weight each year when used over a long period. The shortage of effective
131 and safe drugs in today's medical toolkit highlights the necessity to develop therapeutic agents that
132 have both preventive and treatment capabilities and can be used safely over long durations [35].

Several recent studies suggest that isotopic zinc fractionation plays role in human physiology besides the widespread zinc deficiency problem [36]. The observed zinc isotope fractionation has been attributed to the precipitation of zinc with phytates in the intestine, which inhibits zinc absorption and favors the binding of lighter zinc isotopes [37]. These studies collectively indicate that zinc isotope fractionation occurs during various physiological processes in the human body, including cellular uptake and efflux, intestinal absorption, and incorporation into different tissues. There is no information in the literature that may explain or suggest that isotopic zinc fractionation may or may not be adequately resolved by dietary zinc supplements.

At this time, there is limited direct information about ^{64}zinc excretion in obesity patients specifically. However, normal daily excretion of zinc in urine ranges from 20 to 967 mcg/24 hours in healthy adults [38] while zinc consumption is insufficient. A study analyzing 60 urine samples from 10 healthy participants found that samples with lower zinc concentrations were systematically enriched in heavy zinc isotopes [39]. This is in line with our hypothesis that healthy cellular functions are maintained with light isotopes of zinc.

Given zinc's active involvement in metabolic processes that are closely linked to the pathogenesis of obesity, and the finding that healthy humans excrete heavy zinc isotopes, we have hypothesized that an inherently safe pharmaceutical agent containing enriched light atoms of zinc may render a therapeutic effect on oxidative stress and lipid metabolism, as well as on pancreatic and liver function. This study aims to evaluate the effects of ^{64}Zn- aspartate on the development of obesity induced by a high-fat diet in experimental animals.

2 Methods and Animals

2.1 Investigational Pharmaceutical Agent

^{64}Zn-Aspartate (^{64}Zn-asp coded "KLS-1") is a new complex of isotopically modified zinc and L-aspartate, in which the light zinc ^{64}Zn isotope is enriched to exceed 99% atomic fraction of total zinc. KLS-1 is a small molecule that is structurally a zinc chelate consisting of two molecules of L-aspartic acid and one non-radioactive (stable) atom of ^{64}Zn (Fig.1).

2.2 Animal Model and Experimental Design.

This study utilized white non-linear rats, which were housed in an accredited vivarium at the Educational and Scientific Center "Institute of Biology and Medicine" Taras Shevchenko National University of Kyiv. The animals were cared for by the Standard Rules on the Arrangement, Equipment, and Maintenance of Experimental Biological Clinics (vivariums) and the study adhered to international standards, including the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, 18/03/1986). The study protocol was approved by the Bioethics Commission of the Educational and Scientific Center "Institute of Biology and Medicine" Taras Shevchenko National University of Kyiv.

The study involved 40 rats with an initial body weight of 200±10 g, maintained on a standard diet before the induction of obesity. To model obesity, the rats were fed a high-calorie diet composed of standard feed (60%), lard (10%), chicken eggs (10%), sucrose (9%), peanuts (5%), dry milk (5%), and sunflower oil (1%). The high-calorie diet was prepared in-house. After four weeks on the high-calorie diet, the rats were randomly divided into four groups:

1. Control Group (C): Rats in the control group were fed a standard diet prepared by the vivarium and had free access to water throughout the experiment.

2. Obesity Group (diet-induced obesity, DIO): Rats in this group continued on the high-calorie diet with free access to water for an additional six weeks.

3. Obesity + ^{64}Zn-Asp Group (DIO+^{64}Zn): This group also continued on the high-calorie diet and had free access to water. Additionally, these animals were intragastrically administered a solution of ^{64}Zn- aspartate at a dose of 4.5 mg per animal in a 2 ml solution every third day.

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182 4. Control + ^{64}Zn Group (C+^{64}Zn): Similar to the control group, these rats were fed a standard
183 diet and had free access to water. However, they were also intragastrically administered ^{64}Zn-
184 aspartate at a dose of 4.5 mg per animal in a 2 ml solution every third day.
185 Animals in all groups were weighed once a week following an overnight fast. Daily feed intake
186 was monitored to ensure an accurate assessment of dietary consumption. After a total of 10 weeks on
187 the experimental diets, the animals were sacrificed via decapitation
188 After the experiment, the Body Mass Index (BMI) was calculated for each animal using the ratio
189 of body weight (g) relative to the square of body length (cm^2).
190 **2.3 Preparation of Blood Serum**. Blood serum was prepared from whole blood samples
191 collected from the experimental animals. To remove fibrinogen-related proteins, the blood was
192 incubated at 37°C for 30 minutes. After incubation, a blood clot was carefully dislodged from the
193 walls of the tube using a clean, dry glass rod to expedite serum production. The samples were then
194 centrifuged at 2500 g for 15 minutes. The resulting supernatant (serum) was carefully separated from
195 the blood cells and immediately frozen at -20°C until further analysis.
196 **2.4 Determination of Glucose Concentration in Serum.** Glucose concentration in the
197 blood of animals, fasted for at least 2 hours, was measured using the GLUTOFOT-II glucose meter
198 (LLC "Norma", Ukraine) following the manufacturer's instructions. Blood was drawn from the tail
199 vein using a catheter. The glucose concentration was determined via the glucose oxidase method. The
200 test strip, containing all necessary reagents, facilitated the formation of a colored complex as a result
201 of the reaction. A drop of whole blood was applied to the strip, incubated at room temperature for 30
202 seconds, then washed with distilled water, and analyzed using the glucose meter. Glucose levels were
203 expressed in mmol/L.
204 **2.5 Determination of Serum Alkaline Phosphatase Activity.** Alkaline phosphatase
205 activity in serum was measured spectrophotometrically using a Microlab 300 biochemical analyzer
206 and standard PLIVA-Lachema Diagnostika test kits (Czech Republic). The enzymatic hydrolysis of
207 p-nitrophenyl phosphate by alkaline phosphatase produces p-nitrophenol, which exhibits an intense
208 yellow color in alkaline conditions. The optical density of the samples was measured at 405 nm.
209 Enzyme activity was expressed in relative units.
210 **2.6 Determination of Serum Albumin.** Serum albumin levels were quantified
211 spectrophotometrically using a Microlab 300 biochemistry analyzer and standard PLIVA-Lachema
212 Diagnostika test kits (Czech Republic).
213 **2.7 Determination of Serum Superoxide Dismutase Activity**. Superoxide dismutase
214 (SOD) activity was measured based on the enzyme's ability to inhibit the auto-oxidation of
215 adrenaline. Serum aliquots were added to microplate wells containing 0.2 M bicarbonate buffer, pH
216 10. The reaction was initiated by adding a 0.1% adrenaline solution to each well. The optical density
217 was measured at 347 nm using a μQuant microplate spectrophotometer (BioTek Instruments, USA)
218 at 4 and 8 minutes after the addition of adrenaline. SOD activity was expressed in relative
219 units/min/mg.
220 **2.8 Determination of Catalase Activity.** Catalase activity was assessed using a
221 spectrophotometric method that relies on hydrogen peroxide's ability to form a stable colored
222 complex with molybdenum salts. The reaction was initiated by adding the test sample to 0.03%
223 hydrogen peroxide. After 10 minutes, the reaction was halted by the addition of a 4% ammonium
224 molybdate solution. The optical density was measured at 410 nm using a μQuant microplate
225 spectrophotometer (BioTek Instruments, USA). Catalase activity was quantified using a calibration
226 curve and expressed as μmol H_2O_2/mg protein x min.
227 **2.9 Determination of Diene Conjugates and Schiff Bases in Serum.** To assess the
228 levels of diene conjugates and Schiff bases, aliquots containing 0.1-0.5 mg of protein from the test
229 samples were homogenized in a mixture of heptane and isopropyl alcohol (1:1 ratio) using a tight-
230 fitting glass homogenizer for 10 minutes. The homogenates were then centrifuged at 1000 g for 15

minutes in tightly sealed test tubes. The supernatant was collected, and distilled water was added to separate the heptane and isopropyl alcohol phases. Schiff base levels were determined in the upper heptane phase by measuring the optical density at an excitation wavelength of 360 nm and an emission wavelength of 420 nm using a spectrophotometer. Schiff base concentrations were expressed in units per mg of protein.

For the determination of diene conjugates, an aliquot of the heptane phase was mixed with 96% ethanol, and the optical density was measured at 233 nm using a spectrophotometer SmartSpec (Bio-Rad, USA). The levels of diene conjugates were calculated using a molar extinction coefficient (2.2×10^5 $cm^{-1}\times M^{-1}$) for conjugated dienes formed during the oxidation of polyunsaturated fatty acids and expressed as nmol per mg of protein [13].

2.10 Determination of TBA-Active Products in Serum. The concentration of thiobarbituric acid-reactive substances (TBA-active products) was measured in both serum and adipose tissue homogenates. An aliquot of the test sample was treated with an equal volume of 17% trichloroacetic acid and centrifuged at 1000 g for 15 minutes [13]. The supernatant was then mixed with 0.8% thiobarbituric acid solution and incubated in a boiling water bath for 10 minutes to allow color development. The optical density was measured at 532 nm using a spectrophotometer SmartSpec (Bio-Rad, USA). The concentration of TBA-active products was calculated using a molar extinction coefficient (1.56×10^5 $cm^{-1}\times M^{-1}$) and expressed in nmol per mg of protein.

2.11 Determination of Oxidative Modification of Proteins. The oxidative modification of proteins was assessed by measuring protein carbonyls and Schiff bases through their reaction with 2,4-dinitrophenylhydrazine (DNPH), resulting in the formation of 2,4-dinitrophenylhydrazones of neutral and basic nature [14]. An aliquot containing 0.2 mg of protein was mixed with 0.15 M potassium phosphate buffer (pH 7.4). Proteins were precipitated by adding a 20% TCA solution, and the precipitate was centrifuged at 1000 g for 15 minutes. The precipitate was then treated with 0.1 M DNPH in 2 M HCl and incubated at room temperature for 1 hour. After incubation, the precipitate was washed three times with a 1:1 ethanol: ethyl acetate mixture to remove unbound lipids and DNPH, then dried and dissolved in 8 M urea in a boiling water bath for 10 minutes. The optical density was measured at 356 nm and 370 nm to determine aldehyde and ketone products of oxidative modification, respectively, and was recalculated using appropriate molar extinction coefficients.

2.12 Histopathological Analysis of Pancreatic and Liver Tissues. At the end of the experiment, liver and pancreas samples (0.5×0.5 cm) were immediately placed in a fixative solution (4% paraformaldehyde) at 25°C for 72 hours.

Histological paraffin sections, 5 μm thick, were stained with hematoxylin and eosin. For liver fibrosis assessment, Van Gieson's picro-fuchsin staining method was used. The sections were re-stained with Bömer's hematoxylin, then with Van Gieson's picro-fuchsin, and processed similarly to the hematoxylin-eosin staining. Collagen fibers appeared red, hepatocyte nuclei dark brown, and cytoplasm yellow. The quantitative measuring of red collagen fiber (the related area occupied by collagen fiber) was determined as a percentage of the total tissue area. All histological parameters were analyzed using ImageJ software.

2.13 Statistical Analysis. The distribution of data was assessed with the Shapiro-Wilk normality W-test and variance homogeneity test. One-way ANOVA with Tukey's post hoc multiple comparison tests served for the assessment of significance of the observed changes served for the assessment of the significance of the observed changes. A statistically significant difference was evaluated at $p<0.05$ using Origin 8 Pro. Histograms were created using Microsoft Excel 2010 software (Microsoft, USA) and Origin 8 Pro. The obtained results are presented as mean value ± standard error of the mean (SEM).

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279 3 Results and Discussion

280 3.1 Biochemical and Anthropometric Effects of ^{64}Zn Aspartate in Obesity Animal Models.

281 To evaluate the impact of ^{64}Zn- aspartate form on obesity development induced by high-fat diets,
282 various anthropometric parameters were assessed in obese animal models and in those treated with
283 ^{64}Zn aspartate. The experimental data (**Table 1**) demonstrate that, by the 10th week, the mean body
284 mass index (BMI) of the control animals was 0.60 g/cm², which falls within the reference range for
285 this age group [40]. In contrast, the BMI of animals fed a high-fat diet was 1.14 times higher than
286 that of the control group (0.71 g/cm²). Notably, rats receiving ^{64}Zn aspartate treatment exhibited a
287 lower BMI than the obese animals, but slightly higher BMI than the control values (0.65 g/cm²).
288 These findings suggest that ^{64}Zn-aspartate exerts a beneficial effect on the metabolic status of the
289 obese animals, providing a foundation for further investigation into the mechanisms underlying its
290 effects on obesity.
291 Given that BMI is calculated based on weight, a reduction in BMI may be directly associated
292 with the lower body weight of animals treated with ^{64}Zn- aspartate. Consequently, the effect of ^{64}Zn-
293 aspartate on weight and weight gain in obese animal models was further examined. The experimental
294 data (**Fig. 2**) revealed significant differences in the weight gain dynamics among the experimental
295 groups. Animals on a high-fat diet that received ^{64}Zn- aspartate gained less weight than those fed the
296 high-fat diet only. The most pronounced difference in weight gain between these groups became
297 apparent from the 4th week of the experiment. By the end of the experiment, animals consuming the
298 high-fat diet experienced a 103% increase in body weight, whereas the animals administered
299 intragastric injections of ^{64}Zn- aspartate gained ~62% weight comparable to the control group.
300 Obesity develops due to disruptions in the coordinated functions of various neurotransmitter and
301 hormonal systems, leading to impaired control of appetite and regulation of satiety. This
302 dysregulation promotes excessive food intake and is often accompanied by hyperphagia, a condition
303 marked by an abnormally high desire for food, where the energy intake surpasses the body's energy
304 requirements [41].
305 To explore potential mechanisms underlying the observed decrease in body weight in the animals
306 treated with ^{64}Zn- aspartate, we analyzed the amount of food consumed by the animals (**Table 1).**
307 Both the control group and the obese animals consumed approximately 35 grams of food per day.
308 However, it is important to note that the control group was maintained on a standard diet, whereas
309 the diet-induced obesity (DIO) group was fed a specially prepared high-calorie diet with significantly
310 higher caloric content. A detailed analysis of the results, considering the caloric content of the food
311 consumed, reveals substantial differences. Although the control and DIO groups consumed similar
312 amounts of food, the caloric content of the food in the DIO group was nearly twice that of the control
313 group. Interestingly, animals treated with ^{64}Zn- aspartate consumed lesser amounts of both standard
314 and high-fat diets.
315 The dynamics of the caloric content of food consumed by the animals over the 10-week duration
316 of the experiment are illustrated in **Figure 3**. The data suggest that ^{64}Zn-aspartate influences satiety,
317 as animals treated with this investigational zinc complex consumed significantly less food than the
318 animals maintained solely on a high-fat diet, despite having free access to food. The reduction in
319 food intake, and consequently, the less pronounced weight gain in animals receiving ^{64}Zn- aspartate,
320 as compared to the DIO group, may be attributed to both direct and indirect effects of the isotopically
321 modified zinc on energy homeostasis.
322 In summary, treatment of DIO rats with ^{64}Zn-aspartate resulted in a decrease in daily food
323 consumption, which was accompanied by a less pronounced weight gain and normalization of body
324 mass index compared to untreated obese animals.
325 Zinc deficiency is a key factor in various disorders that, without timely correction and in the
326 presence of specific genetic or metabolic conditions, can lead to the development of several diseases.
327 Therefore, early detection of zinc deficiency is crucial for preventing the onset and progression of

328 metabolic disorders. While plasma zinc levels are commonly used to assess zinc status, they are
329 influenced by many factors and are not always reliable [42].
330 Alternative approaches to determining zinc status include measuring the concentrations of zinc-
331 dependent proteins, particularly enzymes such as carbonic anhydrase, superoxide dismutase, lactate
332 dehydrogenase, and alkaline phosphatase, as well as metallothionein and serum retinol-binding
333 protein. One of the earliest markers of zinc deficiency is the reduced activity of serum alkaline
334 phosphatase and carbonic anhydrase [43]. Zinc deficiency can lead to the development of stress
335 ulcers in the gastrointestinal tract, attributed to a decrease in carbonic anhydrase activity in the
336 mucosa [44].
337 To indirectly assess whether obesity is associated with alterations in zinc status, we measured the
338 alkaline phosphatase activity in blood serum of obese animals and those treated with ^{64}Zn-aspartate.
339 Our study revealed a significant reduction in alkaline phosphatase activity in animals maintained on a
340 high-fat diet (**Table 2**). Specifically, enzyme activity in these animals was 1.5 times lower than in the
341 control group. In contrast, animals treated with ^{64}Zn-aspartate exhibited higher alkaline phosphatase
342 activity compared to both the DIO group and the control group. These findings indirectly confirm
343 zinc deficiency was developed in obese animal models and suggest that treatment with ^{64}Zn-
344 aspartate normalized serum zinc levels.
345 The gastrointestinal tract plays an important role for maintaining zinc homeostasis throughout the
346 body. Zinc absorbed from the intestine enters the bloodstream, where whole blood typically contains
347 approximately 7–8 mg/L of zinc. Notably, about two-thirds of this zinc is transported by red blood
348 cells. In plasma, around 80% of zinc is bound to albumin, with the remaining 20% bound to β2-
349 macroglobulin and transferrin. Published studies confirm a correlation between zinc levels and the
350 concentration of albumin in blood plasma [45].
351 Given the role of albumin in zinc transport, we investigated albumin levels in the untreated obese
352 animal models and the obese animals treated with ^{64}Zn-aspartate. The experimental data indicates
353 that the pathogenesis of obesity is associated with a decrease in serum albumin levels. The
354 administration of ^{64}Zn- aspartate did not significantly affect albumin levels, which remained similar
355 to those in untreated obese animals (**Table 2**).
356 As albumin is the primary transport protein for zinc, a decrease in its concentration could disrupt
357 the timely delivery of zinc to organs such as the liver, where the synthesis of key zinc-containing
358 proteins occurs. This finding aligns with the observed decrease in alkaline phosphatase activity noted
359 earlier.
360 Additionally, ^{64}Zn-aspartate form was found to positively influence lipid metabolism. The levels
361 of triglycerides, cholesterol, and free fatty acids in the serum of animals fed a high-fat diet and
362 treated with Zn-64 stable isotope were nearly comparable to those in the control group.
363 Literature suggests that normal fasting blood glucose levels range from 3.5 to 5.5 mmol/L. An
364 increase in glucose levels to 7.0 mmol/L or higher over time is indicative of hyperglycemia and may
365 predict the development of diabetes mellitus. Our results show that serum glucose levels in the
366 control group and the control group treated with ^{64}Zn- aspartate remained within normal reference
367 values (**Table 3**). The development of obesity led to increase in glucose levels, which were
368 normalized by the administration of ^{64}Zn- aspartate.
369 The glucose-lowering effect of ^{64}Zn- aspartate may be attributed to its ability to stimulate the
370 translocation of glucose transporters from intracellular compartments to adipocyte membranes,
371 thereby enhancing intracellular glucose uptake [46]. Furthermore, ^{64}Zn-aspartate has been shown to
372 increase tyrosine phosphorylation of the insulin receptor β-subunit, improving glucose transport even
373 in the absence of insulin [47]. These findings suggest that ^{64}Zn-aspartate may act as an inhibitor of
374 tyrosine phosphatase-1B, an enzyme that suppresses insulin signaling [48].
375 Given the observed changes in serum glucose levels, we next examined insulin levels. Serum
376 insulin is a critical parameter for diagnosing insulin resistance and prediabetes. In obesity and

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metabolic syndrome, hyperinsulinemia is often a compensatory response to decreased sensitivity of peripheral tissues to insulin, leading to excessive insulin production and secretion by pancreatic β-cells. However, in the later stages of type 2 diabetes mellitus, serum insulin levels decrease significantly due to impaired β-cell function, including reduced insulin production, impaired proinsulin processing, and amyloid deposition in the islets. This β-cell dysfunction further exacerbates the progression of diabetes mellitus [49].

Our study revealed elevated serum insulin levels in obese animals, with a normalizing effect observed in the obese rats treated with ^{64}Zn-aspartate. Interestingly, the administration of ^{64}Zn-aspartate to control group animals also resulted in a slight increase in insulin levels.

Given the critical role of maintaining physiological zinc levels in the body for the synthesis and secretion of insulin, as well as its essential function in pancreatic activity, the effects of ^{64}Zn-aspartate on the overall histophysiology of the pancreas were further investigated.

3.2 Effect Of Stable Isotope ^{64}Zn-64 In the Form Of Aspartate on Histopathological Changes in The Pancreas and Liver of DIO Animal Models.

The pancreas functions as a mixed gland with both exocrine and endocrine components. The majority of the pancreas is composed of exocrine cells organized into acini, which secrete digestive enzymes. These secretions are transported out of the pancreas through a network of intercalated, intralobular, and interlobular ducts, eventually draining into the main pancreatic duct. In the control group (**Fig. 4**), the acini exhibited a typical structure, characterized by granular and brightly acidophilic cytoplasm at the apical pole, and strongly basophilic nuclei at the basal pole.

In contrast, animal models of diet-induced obesity (DIO) (Fig. 4) displayed acini with less pronounced eosinophilic apical cytoplasm (Fig. 4, arrows), a condition likely resulting from lipid accumulation indicative of pancreatic fatty degeneration. However, the administration of ^{64}Zn-aspartate to the rats maintaining standard diet did not alter the morphology of exocrine cells (Fig. 4). Notably, the obese rats treated with ^{64}Zn- aspartate (Fig. 4) exhibited no evidence of fatty degeneration in the pancreatic tissue.

The endocrine component of the pancreas consists of diffusely located islets. A morphometric analysis of the functional state of the endocrine part of the pancreas during the development of induced obesity revealed significant differences among the experimental groups (**Fig. 5**). In the obesity group, the cross-sectional surface area of the islets was markedly reduced by 60%, indicating a substantial decline in the functional activity of the endocrine pancreas. However, in the obese rats treated with ^{64}Zn- aspartate, the cross-sectional surface area of the islets increased by 43% compared to the obesity group, although it remained 29% lower than in the control group. Administration of ^{64}Zn- aspartate to the rats on a standard diet resulted in a noticeable reduction in the cross-sectional surface area of the islets by 39% as compared to the control group.

There exists a direct relationship between the morphological and functional indicators of pancreatic health. The data obtained indicates that the hormone-synthesizing activity of the pancreas in rat models of diet-induced obesity was significantly diminished; however, this activity markedly increased with the administration of ^{64}Zn- aspartate, although it did not fully return to the levels observed in the control group. Based on the observed improvement in islet cross-sectional surface area, it is plausible that a longer or continuous treatment with ^{64}Zn- aspartate could further support islet repair and hormone-synthesizing activity in obese rats. Future studies with extended treatment durations could provide insights into the potential for enhanced regenerative or protective effects on islet architecture. Furthermore, there is evidence of improvement in the exocrine component of the pancreas following the administration of the test substance, as indicated by the disappearance of fatty degeneration, with no significant effects observed in the exocrine cells of rats maintained on a standard diet.

In control rats (**Fig. 6**), the liver exhibited the classical lobular organization, characterized by a central vein running along the axis of each lobule. Hepatocytes, which are polygonal in shape with

426 well-defined nuclei containing several nucleoli, are arranged into ordered hepatic cords radiating
427 from the central vein. Binucleate hepatocytes are also present.
428 In the obesity group (**Fig. 6**), the shape of the hepatocytes transformed from polygonal to rounded
429 due to lipid inclusion deposition, indicative of fatty degeneration of the liver. Additionally, the
430 structure of the hepatic cords becomes disarranged, and the number of binucleate cells in the field of
431 view decreases. Administration of ^{64}Zn-aspartate to the obese rats (**Fig. 6**) restored the structure of
432 the hepatic cords, with most hepatocytes regaining a polygonal morphology and showing no signs of
433 fatty degeneration; binucleate cells were frequently observed. However, the trabecular
434 disorganization remained. The administration of ^{64}Zn-aspartate to rats on a standard diet (**Fig. 6**) did
435 not result in any changes in the morphology of the hepatocytes or the structure of the hepatic lobules.
436 Significant morphometric changes occurred in hepatocytes during the progression of diet-induced
437 obesity (**Fig. 7**). In the obesity group, the nuclear area decreased by 25%, indicating reduced
438 transcriptional activity, which was further evidenced by the nucleus's dark coloration and
439 homogeneous structure, with no nucleoli visible. In contrast, the area of the hepatocytes increased by
440 48% due to substantial lipid inclusion deposition. Consequently, the nucleus-to-cytoplasm ratio
441 decreased significantly (by 45%), reflecting diminished cellular functional activity. Treatment with
442 ^{64}Zn-aspartate improved the morphometric parameters of the obese rats. Specifically, the area of
443 hepatocytes in the animals treated with ^{64}Zn-aspartate decreased by 41% compared to the untreated
444 obesity models, which showed a 13% increase relative to control values, indicating reduced lipid
445 accumulation in hepatocytes. Furthermore, the nucleus-to-cytoplasm ratio increased by 31%
446 compared to the obesity group that showed a reduction of 30% as compared to control. Nevertheless,
447 the nuclear area was reduced by 35% as compared to the control values, indicating a 14% decrease
448 relative to the obesity group, which could be potentially due to the combined effects of a high-fat diet
449 and ^{64}Zn- aspartate on nuclear activity. In rats maintained on a standard diet, ^{64}Zn- aspartate resulted
450 in a reduction of the nucleus area by 26%, the area of hepatocytes by 12%, and the nucleus-to-
451 cytoplasm ratio by 17%.
452 Liver fibrosis is characterized by excessive growth of connective tissue, along with increased
453 synthesis and deposition of collagen in the extracellular matrix. In samples from the control group
454 (**Fig. 8**), most collagen fibers were located in the triads formed by small interlobular vessels.
455 Samples taken from the animals in the obesity group (**Fig. 8**) demonstrated a marked increase in
456 the number of collagen fibers within the triad region, which, as in the control group, consisted of
457 small perilobular capillary plexuses and larger interlobular vessels. Similarly, samples from obese
458 rats injected with ^{64}Zn-aspartate form (**Fig. 8**) revealed comparable levels of collagen fiber deposition
459 in these areas when compared to those from the untreated obesity group. In contrast, the
460 administration of ^{64}Zn- aspartate form to rats on a standard diet (**Fig. 8**) did not lead to significant
461 changes in the quantity of collagen fibers in the perilobular and interlobular capillary plexuses.
462 An analysis of the area occupied by collagen fibers (**Fig. 9**) revealed substantial changes
463 associated with the development of induced obesity. In particular, the area of collagen fiber
464 deposition in the obese group increased by 6.25 times as compared to the control. In the group treated
465 with ^{64}Zn-aspartate, the area of collagen fiber deposition increased by 6 times compared with the
466 control group. No significant differences were observed between the untreated obesity group and the
467 obesity group treated with ^{64}Zn-aspartate. However, the administration of ^{64}Zn-aspartate to rats on a
468 standard diet resulted in a 2-fold increase in the area of collagen fiber deposition.
469 In summary, the findings suggest that ^{64}Zn-aspartate exerts an improved histophysiology of the
470 pancreas and liver in animal models of obesity.

471 ### 3.3 Effects of ^{64}Zn-Aspartate on Prooxidant-Antioxidant Balance in Animal Models
472 ### of Obesity

473 Given the importance of maintaining oxidative homeostasis, we investigated the effects of ^{64}Zn-
474 aspartate on the prooxidant-antioxidant balance in animal models of obesity. Specifically, we

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475 measured the concentrations of primary lipid peroxidation products (LOPs), such as conjugated
476 dienes (CD); secondary products, including TBA-reactive substances (TBARSs); and end products,
477 like Schiff bases (SB) in the blood serum of treated animals.
478 Obesity is typically associated with the development of systemic oxidative stress, which affects
479 most tissues to varying degrees, leading to the disruption of cell membrane integrity and the release
480 of lipid peroxidation products into the bloodstream [50]. Our findings revealed elevated serum levels
481 of primary products of free radical lipid oxidation, with a 1.86-fold increase, suggesting that the
482 initial phase of lipid peroxidation remains active even after ten weeks of induced obesity (**Table 4**).
483 This phenomenon can be attributed to disrupted lipid metabolism, specifically the impaired transport
484 of fatty acids, resulting in increased plasma levels of free and esterified fatty acids, which are direct
485 substrates for reactive oxygen species (ROS) [51]. Additionally, the accumulation of LOPs in serum
486 may be a direct consequence of oxidative damage to cell membranes, allowing these products to
487 permeate into the bloodstream [52].
488 The increase in CD levels was accompanied by the accumulation of secondary LOPs, particularly
489 TBARSs. In obese animals, the serum levels of TBARSs were 4.8 times higher than in control
490 animals. Furthermore, Fe^{2+}-ascorbate-dependent accumulation of TBARSs exceeded control levels
491 by 20-fold, indicating a significant contribution of non-enzymatic reactions in initiating lipid
492 peroxidation, thereby exacerbating the prooxidant-antioxidant imbalance in obesity.
493 The substantial rise in aldehyde LOPs is particularly concerning, as these compounds can bind to
494 proteins, forming stable adducts that may impair protein function. Moreover, proteins modified in
495 this manner may acquire immunological properties, potentially leading to autoantibody production
496 [53].
497 In addition to changes in primary and secondary LOP levels, there was a notable accumulation of
498 the end products of lipid peroxidation, Schiff bases. These are formed through the condensation of
499 aldehydes, such as malondialdehyde, or ketones with protein amino groups, leading to alterations in
500 the structural and functional properties of the proteins. The data indicated a significant increase in
501 Schiff base levels in the serum of obese animals, with values four times higher than those in control
502 animals. The levels of these end products reflect the duration of oxidative stress, and the substantial
503 increase observed suggests prolonged activation of free radical reactions.
504 Elevated levels of lipid peroxidation products after ten weeks of obesity development indicate
505 that oxidative stress is systemic and chronic, which is an unfavorable prognostic marker. These
506 metabolites are highly toxic and contribute to DNA damage, protein and glycosaminoglycan
507 degradation, alterations in cell membrane lipid composition, and disruption of membrane-associated
508 processes.
509 The activation of lipid peroxidation may also indicate increased ROS concentrations, which can
510 activate several serine-threonine kinases, including PKC, AKT/PKB, mTOR, GSK-3, and p38
511 MAPK. These kinases synergistically reduce insulin sensitivity by selectively phosphorylating serine
512 and threonine residues in insulin receptor substrate (IRS) molecules, thereby contributing to the
513 development of insulin resistance in insulin-dependent cells [54].
514 Administration of ^{64}Zn-aspartate form to animals resulted in the normalization of primary,
515 secondary, and end LOP levels, further supporting the ability of ^{64}Zn-aspartate form to modulate the
516 overall prooxidant-antioxidant status of the body.
517 Our studies identified a significant increase in the serum levels of oxidatively modified proteins
518 in animal models of obesity (**Table 5**), with particularly pronounced elevations in aldehyde-
519 dinitrophenyl-hydrazones, indicative of an active phase of oxidative stress and associated metabolic
520 disorders characterized by enhanced free radical formation. The elevated concentrations of carbonyl
521 derivatives in these oxidatively modified proteins, observed alongside intensified lipid peroxidation
522 processes, provide compelling evidence of prolonged oxidative stress in obesity. These findings
523 suggest that the development of obesity is closely associated with the activation of free radical

protein oxidation, as evidenced by the increased formation of carbonyl derivatives with absorption peaks at 356 and 370 nm.

It is important to note that the oxidative modification of protein molecules by reactive oxygen species (ROS) is not confined to pathological conditions. Under physiological conditions, a baseline level of oxidatively modified proteins exists within cells, reflecting a balance between the proteolytic degradation of damaged or "used" molecules and their synthesis [42]. In certain cases, oxidative inactivation serves as a marker stage, enhancing the sensitivity of proteins to proteolytic enzymes, which degrade modified proteins more rapidly than their native counterparts. Therefore, the elevated levels of carbonyl derivatives observed in obese animals may not only signal the presence of oxidative stress but also indicate significant impairments in the regulatory mechanisms governing the degradation of structurally modified proteins, as well as the activity of proteolytic enzymes responsible for this process.

In animals fed a high-fat diet throughout the experiment and treated with ^{64}Zn-aspartate, the levels of aldehyde-dinitrophenyl-hydrazones, while elevated, were lower than those observed in untreated obese animals. In contrast, the concentration of ketone-dinitrophenyl-hydrazones remained within control values. These results correlate with the observed reduction in lipid peroxidation product levels and may suggest a decrease in the intensity of free radical oxidation reactions.

Our experiment revealed a statistically significant decrease in superoxide dismutase (SOD) activity in the untreated obese animals. The administration of ^{64}Zn-aspartate led to an increase in SOD activity, surpassing not only the levels in untreated obese animals, but also the control group animals (**Table 6**). Given that zinc deficiency is often associated with the pathogenesis of obesity, the restoration of SOD activity following treatment with ^{64}Zn- aspartate may indicate normalization of zinc levels in the body and its active role in the regulation and synthesis of zinc-dependent enzymes, particularly SOD.

A decline in SOD activity in the obese animals can be interpreted as a consequence of the depletion of the antioxidant defense system, likely due to the progressive damage of its components by free radicals and lipid peroxidation products (LOPs). According to current understanding, the SOD activity is closely linked to the intensity of LOP processes, as the excessive accumulation of toxic secondary lipid oxidation products can inhibit the activity of SOD and other antioxidant enzymes.

Literature and data analysis on the involvement of ROS in protein oxidative degradation suggest that the reduction in SOD enzymatic activity may result from oxidative modification of the enzyme itself [56]. Since SOD is a metal-containing enzyme, ROS can directly damage the enzyme within its active site. In particular, hydroxyl radicals (OH^{-}), generated via Fenton and Haber-Weiss reactions from hydrogen peroxide and superoxide, act as direct agents that inactivate the enzyme [57].

4 Conclusion

The findings of this study provide a substantial foundation for advocating the use of ^{64}Zn-aspartate as a therapeutic agent in the overweight management of obese patients.

1. It has been demonstrated that administration of ^{64}Zn-aspartate to animals maintained on a high-fat diet resulted in a significant reduction in body mass index, weight, and food intake when compared to the untreated obese animal models.

2. ^{64}Zn-aspartate has been observed to ameliorate histopathological changes in the pancreas and liver of animals subjected to a high-calorie diet, in contrast to untreated obese animal models.

3. The administration of ^{64}Zn-aspartate form has been shown to normalize prooxidant-antioxidant homeostasis in animals fed a high-fat diet. This effect is achieved through a reduction in the intensity of free radical processes, evidenced by decreased levels of lipid peroxidation products and protein

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571 oxidative modification, alongside an enhancement of antioxidant defenses via increased activity of
572 enzymes such as superoxide dismutase and catalase.

573 5 List of non-standard abbreviations:

574 KLS-1 - ^{64}Zn-aspartate

575 DIO - diet-induced obesity

576 TBA-active products - thiobarbituric acid-reactive substances

577 TCA –

578 DNPH - 2,4-dinitrophenylhydrazine

579 BMI - body mass index

580 LOPs - lipid peroxidation products

581 TBARSs - TBA-reactive substances

582 SB - Schiff bases

583 CD - conjugated dienes

584 SOD - superoxide dismutase
585
586

587 6 Conflict of Interest

588 The authors declare that the research was conducted in the absence of any commercial or financial
589 relationships that could be construed as a potential conflict of interest.

590 7 Author Contributions

591 MT: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project
592 administration, Software, Supervision, Validation, Visualization, Writing–original draft, Writing–
593 review and editing. SG: Formal Analysis, Investigation, Methodology, Project administration,
594 Software, Validation, Writing–review and editing. AB: Conceptualization, Formal Analysis, Project
595 administration, Supervision, Validation, Writing–review and editing, Data curation, Investigation,
596 Methodology, Software. RB: Data curation, Formal Analysis, Investigation, Software, Supervision,
597 Writing–review and editing. OK: Data curation, Formal Analysis, Investigation, Project
598 administration, Software, Visualization, Writing–original draft, Writing–review and editing. TV:
599 Data curation, Formal Analysis, Investigation, Methodology, Project administration, Software,
600 Writing–review and editing. TH: Investigation, Software, Writing–review and editing, Data curation.
601 NR: Data curation, Investigation, Software, Writing–review and editing. TF: Data curation,
602 Investigation, Methodology, Software, Visualization, Writing–review and editing. OS:
603 Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Supervision,
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770

771 **Tables:**

772 **Table 1:** Anthropometric Values, Food Intake, and Caloric Content (M ± SEM, n = 10)

	Experimental groups			
	C	C+zinc	DIO	DIO+zinc
BMI (g/cm^2)	0.60±0.004	0.59±0.001	0.71±0.002*	0.65±0.001$^{*\#}$
Weight gain as of the end of the experiment (%)	59±5	59±6	103±11*	62±7$^{\#}$
Amount of food consumed (g/day)	34±3	32±2	35±1	29±2$^{\#}$
Caloric content of food (kJ/day)	525±45	490±32	1001±54*	823±21$^{*\#}$

773 **Note:**

774 – **C**: Control group
775 – **C + Zinc**: Control group treated with Zn-64 stable isotope in aspartate form
776 – **DIO**: Diet-induced obesity group

777 – **DIO + Zinc**: Diet-induced obesity group treated with Zn-64 stable isotope in aspartate form

778 * - the difference is significant compared to the control group of animals;

779 # - the difference is significant compared to the group of animal models of obesity.

780 **Table 2:** Biochemical analysis of blood serum of experimental animals (M±SEM, n=10)

781

parameters \ groups	C	DIO	DIO+zinc
Alkaline phosphatase activity, CU	74.3±12.1	37.2±15.4*	87.6±18.7#
Albumin levels, CU	219.2±14.6	168.8±16.8*	166.2±15.8*
Triglycerides, g/L	2.55±0.20	4.39±0.73*	2.79±0.30#
Cholesterol, mmol/L	2.42±0.19	5.76±0.87*	2.83±0.23#
Free fatty acids, mg/L	23.60±4.67	74.50±9.23*	31.62±7.92#

782 CU - conditional units;

783 * - the difference is significant compared to the control group of animals;

784 # - the difference is significant compared to the group of animal models of obesity.

785 **Table 3:** Serum glucose concentration and insulin level in experimental animals (M±SEM, n=10)

Experimental groups	Insulin levels, CU	Glucose levels, mmol/L
C	0.133 ± 0.024	4.4 ± 0.3
C+zinc	0.145 ± 0.013	4.7 ± 0.2
DIO	0.216 ± 0.035*	7.1 ± 0.1*
DIO+zinc	0.149 ± 0.018#	4.9 ± 0.2#

786 CU - conditional units;

787 * - the difference is significant compared to the control group of animals;

788 # - the difference is significant compared to the group of animal models of obesity.

789 **Table 4:** Level of lipid peroxidation products in the blood serum of animals in the experimental
790 groups (M±SEM, n=10)

		TBA-reactive substances,	

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Experimental groups	Conjugated dienes, nmol/mg protein	nmol/mg protein		Schiff bases, CU/mg protein
		Spontaneous accumulation	Fe^{2+}-ascorbate-induced accumulation	
C	0.021±0.001	0.006±0.0003	0.033±0.005	41.31±2.47
DIO	0.039±0.002 *	0.029±0.002 *	0.61±0.003 *	168.86±8.15 *
DIO+zinc	0.025±0.008	0.005±0.0003 #	0.15±0.008 *#	56.27±4.33 *#

791 CU - conditional units; TBA - Thiobarbituric Acid;

792 * - the difference is significant compared to the control group of animals;

793 # - the difference is significant compared to the group of animal models of obesity.

794

795 **Table 5:** Level of oxidative modification products of proteins in the blood serum of animals of
796 experimental groups (M±SEM. n=10)

Groups	Aldehyde-dinitrophenyl-hydrazones. nmol/mg protein	ketone-dinitrophenyl-hydrazones. nmol/mg protein
C	0.187±0.009	0.255±0.023
DIO	0.698±0.041 *	0.571±0.035 *
DIO+zinc	0.253±0.012 *#	0.200±0.024 *#

797 * - the difference is significant compared to the control group of animals;

798 # - the difference is significant compared to the group of animal models of obesity.

799

800

801 **Table 6:** Superoxide dismutase and catalase activity in the blood serum of animals in the
802 experimental groups (M±SEM. n=10)

803

	Experimental groups		
	C	DIO	DIO+zinc
Superoxide dismutase activity. CU/ min per mg protein	3.36±0.36	2.65±0.41 *	4.5±0.43 *#
Catalase activity.	0.52±0.05	0.43±0.02*	0.48±0.02#

μmol H_2O_2/min per mg protein			

804 CU - conditional units;

805 * - the difference is significant compared to the control group of animals;

806 # - the difference is significant compared to the group of animal models of obesity.

807

808 **Figures:**

809 **Fig. 1.** Schematic Representation of KLS-1 Structure

810 **Fig. 2.** Dynamics of body weight gain in animals in experimental groups (M ± SEM, n=10)

811 **Fig. 3.** Caloric content of food consumed by animals of experimental groups (M ± SEM. n=10)

812 Note: 1- Control;

813 2- Control+Zn-64 stable isotope in aspartate form (C+Zinc);

814 3 - Obesity+Zn-64 stable isotope in aspartate form (DIO+Zinc);

815 4 - Obesity (DIO).

816 **Fig. 4.** Microphotographs of the pancreas of rats from experimental groups: control (A, B, C), DIO -
817 Diet-induced obesity group (D, E, F), DIO+Zinc - Diet-induced obesity group treated with Zn-64
818 stable isotope in aspartate form (G, H, I), Control+Zinc - Control group treated with Zn-64 stable
819 isotope in aspartate form (J, K, L). B, E, H, K - endocrine part of pancreas; C, F, I, L – exocrine part
820 of pancreas.

821 Hematoxylin and eosin staining. Arrow - lipid dysthrophic accumulations, scale bar 100 μm.

822 **Fig. 5.** Cross-sectional area of the pancreatic islets. Data are presented as the mean value ± SEM. * -
823 the difference is significant compared to the control group of animals;

824 # - the difference is significant compared to the group of animal models of obesity.

825 **Fig. 6.** Microphotographs of the liver of rats from experimental groups: control (A, B), DIO - Diet-
826 induced obesity group (C, D), DIO+Zinc - Diet-induced obesity group treated with Zn-64 stable
827 isotope in aspartate form (E, F), Control+Zinc - Control group treated with Zn-64 stable isotope in
828 aspartate form (G, H). Hematoxylin and eosin staining, scale bar 100 μm.

829 **Fig. 7.** Morphometric analysis (cross-sectional area of hepatocyte nuclei, cross-sectional area of
830 hepatocyte, nuclear-cytoplasmic ratio of hepatocytes) of the liver. Data are presented as the mean
831 value ± SEM.

832 * - the difference is significant compared to the control group of animals;

833 # - the difference is significant compared to the group of animal models of obesity.

834 **Fig. 8.** Microphotographs of the liver of rats from experimental groups: control (A, B), DIO - Diet-
835 induced obesity group (C, D), DIO+Zinc - Diet-induced obesity group treated with Zn-64 stable
836 isotope in aspartate form (E, F), Control+Zinc - Control group treated with Zn-64 stable isotope in
837 aspartate form (G, H). Van Gieson’s staining method for the detection of collagen fibers (red color),
838 scale bar 100 μm.

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839 **Fig. 9.** Fibrosis level in liver of of rats from experimental groups. Data are presented as the mean
840 value ± SEM. * - the difference is significant compared to the control group of animals;
841 # - the difference is significant compared to the group of animal models of obesity.
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