e-ISSN 1734-9168
http://www.isez.pan.krakow.pl/en/folia-biologica.html

Folia Biologica (Kraków), vol. 72 (2024), No 1
https://doi.org/10.3409/fb_72-1.04

Dietary zinc deficiency increases damage rate and copy number of mitochondrial DNA in the mouse liver

Katarzyna Kotarska

Accepted March 22, 2024 *Published online March 29, 2024* *Issue online March 29, 2024*

Short communication

Kotarska K. 2024. Dietary zinc deficiency increases damage rate and copy number of mitochondrial DNA in the mouse liver. Folia Biologica (Kraków) **72**: 29-34.

Zinc is an essential trace element involved in many physiological processes, ranging from cellular growth and immune functions to enzymatic activity and gene expression. Inadequate dietary zinc levels can disrupt the functioning of numerous organs, including the liver. Given the pivotal role played by the liver, in-depth understanding of the mechanisms driving disorders in this organ caused by zinc deficiency is of great importance. In the present study, the effects of a four-week low-zinc diet on the integrity and copy number of mitochondrial DNA (mtDNA) in the liver was assessed using a mouse model. The research revealed a significantly elevated damage rate and an increased copy number of mtDNA in the livers of mice subjected to a low-zinc diet when compared to control animals. These findings indicate that a zinc deficiency, by promoting DNA damage in mitochondrial genomes, increases a potential risk of harmful mutations that could compromise ATP production in the liver. The rise in the mtDNA copy number suggests an initial compensatory response to the detrimental effects of the zinc deficiency, which is likely to diminish with a chronic insufficiency of this element. The study confirmed the significant role of mitochondria in the processes leading to liver dysfunction induced by a zinc deficiency. It showed additionally that mtDNA is a very sensitive indicator of the liver's condition that is responsive to environmental changes such as a micronutrient deficiency in the diet.

Key words: low-zinc diet, mtDNA lesions, mtDNA quantity.

Katarzyna Kotarska✉, Laboratory of Genetics and Evolution, Institute of Zoology and Biomedical Research, Department of Biology, Jagiellonian University, Kraków, Poland.
E-mail: katarzyna.kotarska@uj.edu.pl

In contemporary societies, there is an alarming trend towards consuming excessively caloric meals, replete with saturated fats and simple sugars. This dietary pattern contributes to the development of hypercholesterolemia, insulin resistance, obesity and the associated complications. Simultaneously, the diets of many individuals lack micronutrients essential for the proper functioning of the body. Among these micronutrients, zinc (Zn) stands out as a vital component of numerous enzymatic systems and transcription factors. Zinc fulfils a multitude of functions; therefore, its insufficiency in the diet can impair the physiological processes of various tissues and organs (Prasad 2004). The liver is one of the organs that is particularly susceptible to a zinc deficiency. Extensive research has established a clear connection between an inadequate zinc intake and chronic liver dysfunctions, as well as a diminished capacity for its regeneration (Grüngreiff *et al*. 2016; Martinez *et al.* 2017; Barbara & Mindikoglu 2021). Moreover, zinc supplementation has shown promising results in the treatment of several severe liver conditions, including liver cirrhosis, fatty liver disease and hepatic encephalopathy (Shen *et al.* 2019; Bloom *et al.* 2021). Therefore, it is of great significance to comprehensively investigate both the role

of zinc in the liver's optimal functioning and the contribution of its deficiency to the pathophysiology of liver diseases.

In the present study, I evaluated the effects of zinc deficiency on the integrity and copy number of mitochondrial DNA (mtDNA) in the liver, using a mouse model. To date, the impact of a zinc deficiency on the liver mitochondria has primarily been explored within the context of alcoholism. Both people affected by alcoholism and animal models of this condition exhibit a significant reduction of zinc levels in the serum and liver tissues (Zhou *et al.* 2005). Moreover, it has been demonstrated that rats subjected to chronic alcohol consumption display a reduced zinc content within their liver mitochondria, accompanied by impaired respiratory chain functions. These disturbances lead to a decrease in ATP production and an excessive concentration of reactive oxygen species (ROS) within the hepatocytes (Sun *et al.* 2015; 2016). The above research led to the assumption that mitochondrial dysfunctions resulting from chronic alcohol consumption are directly linked to a decreased zinc concentration. Consequently, the reduced zinc concentration is a pivotal mediator of the pathological alterations observed in alcoholism (Zhou *et al.* 2005; Sun *et al.* 2015; 2016). This hypothesis is substantiated by a study indicating that zinc supplementation during ethanol treatment effectively safeguards mice against liver damage (Zhou *et al.* 2005). However, it is worth noting that in the case of alcoholism, disruptions to the structure and functions of the liver mitochondria may arise not only from a zinc deficiency, but also from other factors associated with a long-term exposure to ethanol. The current investigation allowed me to assess the genome integrity and quantity of liver mitochondria, where a zinc deficiency was exclusively induced by a diet deficient in this essential element. The zinc deficiency in the employed model was the primary, not the secondary, cause of the observed mitochondrial changes, which provided a new insight into the subject. This subject is very important, considering the fact that a dietary zinc deficiency is an acute problem and a serious health risk factor in modern human populations (Knez & Stangoulis 2023).

Materials and Methods

Animals

The experiments were conducted on 7-week-old male C57BL/6J mice (Charles River, Germany) maintained in standard laboratory conditions (12 h light-dark cycle, 19-21°C), with free access to tap water and food. After a one-week acclimation, the mice were subjected for 4 weeks to either a zinc-adequate feed (control, 50 mg/kg) or a zinc-deficient feed (C1040, Altromin, Germany) (ZnD, 2 mg/kg). Following that, the mice were euthanised *via* decapitation, and their livers were isolated and frozen at -80°C for further analysis. The liver tissues (from 7 control and 7 ZnD mice) were collected during the implementation of another research project conducted at the Maj Institute of Pharmacology, Polish Academy of Sciences, in accordance with the EU Directive 2010/63/EU and with the approval of the local ethics commission (Permission Number: 87/2021). The 4-week low-zinc diet employed in this study leads to a significant decrease in zinc levels, both in the serum and the internal organs, as was demonstrated previously in rats (Doboszewska *et al.* 2016) and mice (Pochwat *et al.* 2022).

DNA extraction

The liver tissues (20 mg) were incubated overnight in 350 µl of lysis buffer with RNase A (10 mg/ml, 2 µl) and proteinase K (20 mg/ml, 20 µl). Next, DNA extraction from the obtained lysates was performed using the Tissue DNA Purification Kit (EURx, Poland). DNA samples were measured in a Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific, USA) and were then diluted with sterile water to a concentration of 0.5 ng/µl.

Quantification of lesions in the mtDNA

The number of lesions in the liver mtDNA was assessed with long-run real-time PCR-based DNA-damage quantification (LORD-Q) using primers amplifying a long fragment (3255 bp) and an internally nested short fragment (78 bp) of the D-loop region. The primer sequences were as follows: forward long – 5'-AGCTACCCCCAAGTTTAATGG-3'; reverse long – 5'-TCCTACTGGTCCGATTCCAC-3'; forward short – 5'-CGAGAAGAGGGGCATTGGTG-3'; and reverse short – 5'-ATCCCCTTCCCCATTTGGTC-3'. The long fragment, in which the amplification was proportional to the number of lesions in the DNA template, served as an experimental probe. The short fragment, whose amplification did not depend on the degree of DNA integrity, served as a normalisation control (Lehle *et al.* 2014; Dannenmann *et al.* 2017). Taq polymerase and standard SYBR Green I dye were used for the amplification and real-time detection of the short sequence. In order to ensure a proper amplification of the long sequence, high-fidelity and

rapid PrimeSTAR GXL polymerase was employed in combination with ResoLight dye, which is less inhibitory to DNA polymerase during a long-run PCR.

The reaction mixture for the short fragment included: 2 µl of DNA template, PowerUp SybrGreen Master Mix (Applied Biosystems, USA) and 200 nM of each primer in a total volume of 10 µl per well. The real-time analysis was performed in the QuantStudio 5 Real-Time PCR System (Applied Biosystems). The cycling conditions were as follows: 1) 2 min at 50°C; 2) 2 min at 95°C; 3) 40 cycles of 15 s at 95°C, and 1 min at 60°C; and 4) melt curve drawing. The reaction mixture for the long fragment included: 2 µl of DNA template, 0.4 µl of PrimeSTAR GXL Polymerase (Takara, Japan), PrimeSTAR GXL Buffer (Takara), dNTP mix (200 µM each) (Takara), primers (200 nM each) and 0.05 of ResoLight Dye (Roche, Switzerland) in a total volume of 10 µl per well. The real-time analysis was carried out on the LightCycler 96 System (Roche). The cycling conditions were as follows: 1) 1 min at 98°C; 2) 40 cycles of 10 s at 98°C, 15 s at 60°C, and 35 s at 68°C; and 3) melt curve drawing. All of the DNA samples were run in triplicate. In each 96-well PCR plate, apart from the samples, serial two-fold dilutions of standard DNA were amplified to assess the reaction efficiency. Mean Ct values for the amplification of the long and short fragments, together with the amplification efficiency, were entered into the formula introduced by Lehle *et al.* (2014), which is based on the comparative ΔΔCt method and allows the number of DNA lesions per 10 kb to be calculated. DNA damage in the examined samples was calculated in relation to the control samples, which were assumed to be damage-free.

Quantification of the mtDNA copy number

In order to assess the number of mtDNA copies per cell of liver tissue, the real-time PCR amplification of the short mtDNA fragment (performed during LORD-Q assay) was normalised against the amplification of a single copy nuclear gene *ApoB* (performed for the purpose of the current analysis on the same DNA samples). The primers amplifying the 74 bp fragment of the *ApoB* gene were as follows: forward – 5'-CACGTGGGCTCCAGCATT-3'; and reverse – 5'-TCACCAGTCATTTCTGCCTTTG-3' (Machado *et al.* 2015). The reaction mixture included: 2 µl of DNA template, PowerUp SybrGreen Master Mix (Applied Biosystems) and 200 nM of each primer in a total volume of 10 µl per well. The real-time analysis was performed in the QuantStudio 5 Real-Time PCR System (Applied Biosystems). The cycling conditions were as follows: 1) 2 min at 50°C; 2) 2 min at 95°C; 3) 40 cycles of 15 s at 95°C, and 1 min at 60°C; and 4) melt curve drawing. All the DNA samples were run in triplicate. Mean Ct values for the amplification of the nuclear *ApoB* gene and mitochondrial D-loop sequence were used to calculate the number of mtDNA copies per cell utilising the $2 \times 2^{\Delta Ct}$ formula (Rooney *et al.* 2015).

Statistics

A statistical analysis of the results was conducted using the GraphPad Prism. The initial step involved assessing the normal distribution of the values within groups. To achieve this, the Shapiro-Wilk normality test was employed, revealing that all the datasets met the criteria of a normal distribution and consequently could be subjected to a parametric analysis. The significance of differences between the experimental group (zinc deficit) and the control group was therefore verified using the parametric Student's t-test. The level of significance was set to $p<0.05$.

Results and Discussion

Zinc is an essential antioxidant, serving as cofactor or a structural element in numerous enzymes responsible for neutralising reactive oxygen species (ROS). A deficiency in zinc is known to cause oxidative stress, which is characterised by an imbalance between ROS production and their neutralisation (Song *et al.* 2009; Sharif *et al.* 2012; Martinez *et al.* 2017). The oxidative stress, in turn, can lead to DNA damage, particularly affecting mtDNA which, due to its location in close proximity to the respiratory chain and the absence of protective histones, is very susceptible to the deleterious activity of ROS (Guo *et al.* 2013; Liu & Chen 2017). Given that zinc plays an essential role in ROS neutralisation, and an excessive ROS level promotes DNA damage, I hypothesised that mitochondria in the livers of mice subjected to a zinc-deficient diet would have a more damaged genome than the mitochondria in the livers of mice receiving standard feed. Indeed, the real-time PCR analyses in this study showed that the animals on a zinc-deficient diet exhibited a significantly higher number of lesions in their liver mtDNA compared to the control group (Fig. 1). The elevated level of mtDNA damage, coupled with the compromised nucleic acid repair systems observed in cases of zinc deficiency (Song *et al.* 2009; Sharif *et al.* 2012; Sharma *et al.* 2024), may result in mutations that detrimentally affect the mitochondrial functions. The abnormalities in mitochondrial functions, and the associated

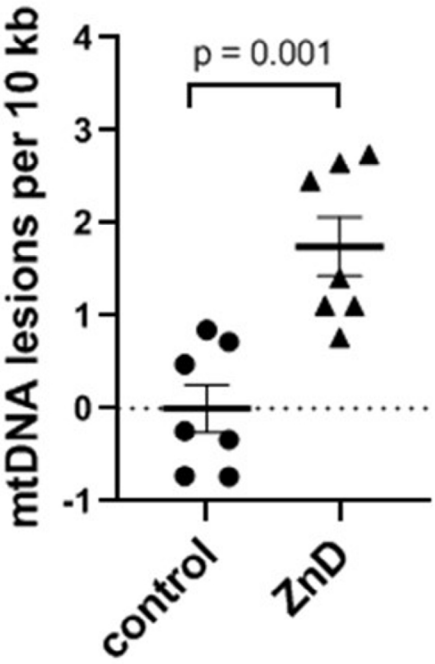

Fig. 1. Number of mtDNA lesions in the livers of mice subjected to a zinc-deficient diet (ZnD) in relation to mice fed with a zinc-adequate diet (control). The graph shows individual values (represented by circles and triangles), as well as the means for both groups (illustrated by horizontal lines), with error bars denoting the standard errors. The p-value was calculated using a t-test. N=7.

which serves as a pivotal antioxidant defender within eukaryotic cells (Eleutherio *et al.* 2021).

Liver diseases are frequently associated not only with structural impairments and dysfunctions of the mitochondria, but also with substantial alterations in the quantity of these organelles within hepatocytes, commonly estimated through the counting of mtDNA copies (Chiappini *et al.* 2006; Pirola *et al.* 2015; Kamfar *et al.* 2016; McKiernan *et al.* 2016). Research has demonstrated that a zinc deficiency caused by prolonged alcohol consumption results in impaired mitochondrial biogenesis and, subsequently, in a decrease in the number of mtDNA copies in the liver tissue (Sun *et al.* 2016). Intriguingly, the analyses carried out in the current study revealed a significant increase in mtDNA copies in the livers of mice subjected to a zinc-deficient diet compared to control animals (Fig. 2). I suggest that this observed rise in mitochondrial abundance under a dietary zinc deficiency may be indicative of a compensatory mechanism. As a zinc deficiency compromises the respiratory chain efficiency (Sun *et al.* 2015; 2016), heightened mitochondrial biogenesis could serve as a protective response, preventing a decline in energy production within the liver. It is crucial to note that my research model involved subjecting mice to a low-zinc diet for a relatively short period of four

reduction in energy metabolism in hepatocytes, are factors that commonly contribute to diverse types of liver diseases (Grattagliano *et al.* 2011; Pirola *et al.* 2021). Hence, the documented rise in the number of mtDNA lesions in the livers of mice on a low-zinc diet is a significant stride toward comprehending the mechanisms underlying zinc deficiency-induced disruptions in the functionality of this vital organ. The outcome simultaneously underscores the critical role of zinc in mitigating oxidative stress and in preserving the integrity of mitochondrial genomes. Regrettably, the LORD-Q method employed in the study lacks the capability to differentiate between various types of DNA damage and is unable to detect lesions that do not impede the PCR efficiency (Kotarska *et al.* 2024). Consequently, future investigations should aim to quantify the distinct lesion types within hepatic mtDNA, such as 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG), to elucidate their prevalence under the conditions of zinc deficiency. Additionally, comprehensive analyses could encompass an assessment of the zinc-dependent enzyme activity involved in ROS neutralisation, with a particular emphasis on superoxide dismutase (SOD1),

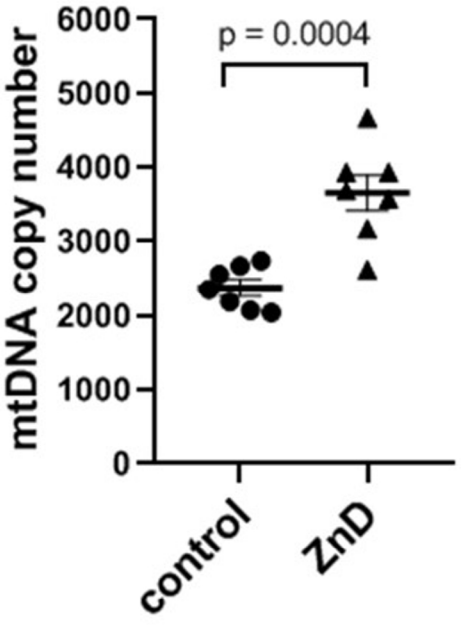

Fig. 2. Number of mtDNA copies in the livers of mice subjected to a zinc-deficient diet (ZnD) and mice fed with a zinc-adequate diet (control). The graph shows individual values (represented by circles and triangles), as well as the means for both groups (illustrated by horizontal lines), with error bars denoting the standard errors. The p-value was calculated using a t-test. N=7.

weeks. This brief duration of zinc deficiency might have elicited a robust initial defence response from the body. I hypothesise that a prolonged exposure to zinc deficiency could lead to a disappearance of the initial increased mitochondrial count in the livers of mice, or could even lead to a significant reduction of this parameter, as is suggested by the data in the existing literature (Sun *et al.* 2016; Filograna *et al.* 2021). Nevertheless, the obtained result proves that the mtDNA copy number is a highly sensitive indicator of the liver's condition that is responsive to environmental changes such as a micronutrient deficiency in the diet.

In summary, the current research revealed an elevated number of lesions in mitochondrial genomes within the livers of mice fed with a low-zinc diet. This outcome suggests that a zinc deficiency, coupled with the associated oxidative stress, may induce DNA damage in the liver mitochondria, potentially resulting in deleterious mutations and an impaired functionality of these vital organelles. The findings also indicated an increase in the mtDNA copies within the livers of zinc-deficient mice compared to control animals. This phenomenon appears to represent an initial defensive response by the body to mitigate the adverse effects of the zinc deficiency, and might diminish with a chronic insufficiency of this element. The study's results underscore the significant role played by mitochondria in the processes leading to liver dysfunction induced by a zinc deficiency. However, a comprehensive understanding of this role necessitates further in-depth analyses.

Acknowledgments

The author would like to thank Prof. Bernadeta Szewczyk from the Maj Institute of Pharmacology, Polish Academy of Sciences, Laboratory of Trace Elements Neurobiology, Krakow, Poland for providing the mouse liver tissues utilised in the analysis carried out in this study.

Author Contributions

Research concept and design, Collection and/or assembly of data, Data analysis and interpretation, Writing the article, Critical revision of the article, Final approval of article – K.K.

Conflict of Interest

The author declares no conflict of interest.

References

Barbara M., Mindikoglu A.L. 2021. The role of zinc in the prevention and treatment of nonalcoholic fatty liver disease. Metabol. Open. **11**: 100105. https://doi.org/10.1016/j.metop.2021.100105

Bloom A., Bloom S., Silva H., Nicoll A.J., Sawhney R. 2021. Zinc supplementation and its benefits in the management of chronic liver disease: An in-depth literature review. Ann. Hepatol. **25**: 100549. https://doi.org/10.1016/j.aohep.2021.100549

Chiappini F., Barrier A., Saffroy R., Domart M.C., Dagues N., Azoulay D., Sebagh M., Franc B., Chevalier S., Debuire B., Dudoit S., Lemoine A. 2006. Exploration of global gene expression in human liver steatosis by high-density oligonucleotide microarray. Lab. Invest. **86**: 154-165. https://doi.org/10.1038/labinvest.3700374

Dannenmann B., Lehle S., Lorscheid S., Huber S.M., Essmann F., Schulze-Osthoff K. 2017. Simultaneous quantification of DNA damage and mitochondrial copy number by long-run DNA-damage quantification (LORD-Q). Oncotarget. **8**: 112417-112425. https://doi.org/10.18632/oncotarget.20112

Doboszewska U., Szewczyk B., Sowa-Kućma M., Noworyta-Sokołowska K., Misztak P., Gołębiowska J., Młyniec K., Ostachowicz B., Krośniak M., Wojtanowska-Krośniak A., Gołembiowska K., Lankosz M., Piekoszewski W., Nowak G. 2016. Alterations of bio-elements, oxidative, and inflammatory status in the zinc deficiency model in rats. Neurotox Res. **29**: 143-154. https://doi.org/10.1007/s12640-015-9571-7

Eleutherio E.C.A., Silva Magalhães R.S., de Araújo Brasil A., Monteiro Neto J.R., de Holanda Paranhos L. 2021. SOD1, more than just an antioxidant. Arch Biochem Biophys. **697**: 108701. https://doi.org/10.1016/j.abb.2020.108701

Filograna R., Mennuni M., Alsina D., Larsson N.G. 2021. Mitochondrial DNA copy number in human disease: the more the better? FEBS Lett. **595**: 976-1002. https://doi.org/10.1002/1873-3468.14021

Grattagliano I., Russmann S., Diogo C., Bonfrate L., Oliveira P.J., Wang D.Q., Portincasa P. 2011. Mitochondria in chronic liver disease. Curr. Drug Targets **12**: 879-893. https://doi.org/10.2174/138945011795528877

Grüngreiff K., Reinhold D., Wedemeyer H. 2016. The role of zinc in liver cirrhosis. Ann. Hepatol. **15**: 7-16. https://doi.org/10.5604/16652681.1184191

Guo C., Sun L., Chen X., Zhang D. 2013. Oxidative stress, mitochondrial damage and neurodegenerative diseases. Neural. Regen. Res. **8**: 2003-2014. https://doi.org/10.3969/j.issn.1673-5374.2013.21.009

Kamfar S., Alavian S.M., Houshmand M., Yadegarazari R., Seifi Zarei B., Khalaj A., Shabab N., Saidijam M. 2016. Liver mitochondrial DNA copy number and deletion levels may contribute to nonalcoholic fatty liver disease susceptibility. Hepat. Mon. **16**: e40774. https://doi.org/10.5812/hepatmon.40774

Knez M., Stangoulis J.C.R. 2023. Dietary Zn deficiency, the current situation and potential solutions. Nutr Res Rev. **36**: 199-215. https://doi.org/10.1017/S0954422421000342

Kotarska K., Gąsior Ł., Rudnicka J., Polański Z. 2024. Long-run real-time PCR analysis of repetitive nuclear elements as a novel tool for DNA damage quantification in single cells: an approach validated on mouse oocytes and fibroblasts. J. Appl .Genet. **65**: 181-190. https://doi.org/10.1007/s13353-023-00817-0

Lehle S., Hildebrand D.G., Merz B., Malak P.N., Becker M.S., Schmezer P., Essmann F., Schulze-Osthoff K., Rothfuss O. 2014. LORD-Q: A long-run real-time PCR-based DNA-damage quantification method for nuclear and mitochondrial genome analysis. Nucleic Acids Res. **42**: e41. https://doi.org/10.1093/nar/gkt1349

Liu X., Chen Z. 2017. The pathophysiological role of mitochondrial oxidative stress in lung diseases. J. Transl. Med. **15**: 207. https://doi.org/10.1186/s12967-017-1306-5

Machado T.S., Macabelli C.H., Sangalli J.R., Rodrigues T.B., Smith L.C., Meirelles F.V., Chiaratti M.R. 2015. Real-time PCR quantification of heteroplasmy in a mouse model with mitochondrial DNA of C57BL/6 and NZB/BINJ strains. PLoS One. **10**: e0133650. https://doi.org/10.1371/journal.pone.0133650

Martinez S.S., Campa A.L.Y., Fleetwood C., Stewart T., Ramamoorthy V., Baum M.K. 2017. Low plasma zinc is associated with higher mitochondrial oxidative stress and faster liver fibrosis development in the Miami adult studies in HIV cohort. J. Nutr. **147**: 556-562. https://doi.org/10.3945/jn.116.243832

McKiernan P., Ball S., Santra S., Foster K., Fratter C., Poulton J., Craig K., McFarland R., Rahman S., Hargreaves I., Gupte G., Sharif K., Taylor R.W. 2016. Incidence of primary mitochondrial disease in children younger than 2 years presenting with acute liver failure. J. Pediatr. Gastroenterol. Nutr. **63**: 592-597. https://doi.org/10.1097/MPG.0000000000001345

Pirola C.J., Garaycoechea M., Flichman D., Castaño G.O., Sookoian S. 2021. Liver mitochondrial DNA damage and genetic variability of Cytochrome b - a key component of the respirasome - drive the severity of fatty liver disease. J. Intern. Med. **289**: 84-96. https://doi.org/10.1111/joim.13147

Pirola C.J., Scian R., Gianotti T.F., Dopazo H., Rohr C., Martino J.S., Castaño G.O., Sookoian S. 2015. Epigenetic modifications in the biology of nonalcoholic fatty liver disease: The role of DNA hydroxymethylation and TET proteins. Medicine (Baltimore). **94**: e1480. https://doi.org/10.1097/MD.0000000000001480

Pochwat B., Misztak P., Masternak J., Bączyńska E., Bijata K., Roszkowska M., Bijata M., Włodarczyk J., Szafarz M., Wyska E., Muszyńska B., Krakowska A., Opoka W., Nowak G., Szewczyk B. 2022. Combined hyperforin and lanicemine treatment instead of ketamine or imipramine restores behavioral deficits induced by chronic restraint stress and dietary zinc restriction in mice. Front Pharmacol. **13**: 933364. https://doi.org/10.3389/fphar.2022.933364

Prasad A.S. 2004. Zinc deficiency: its characterization and treatment. Met. Ions Biol. Syst. **41**: 103-137.

Rooney J.P., Ryde I.T., Sanders L.H., Howlett E.H., Colton M.D., Germ K.E., Mayer G.D., Greenamyre J.T., Meyer J.N. 2015. PCR based determination of mitochondrial DNA copy number in multiple species. Methods Mol. Biol. **1241**: 23-38. https://doi.org/10.1007/978-1-4939-1875-1_3

Sharif R., Thomas P., Zalewski P., Fenech M. 2012. The role of zinc in genomic stability. Mutat. Res. **733**: 111-121. https://doi.org/10.1016/j.mrfmmm.2011.08.009

Sharma P., Wong C.P., Ho E., Sampath H. 2024. Catalytic activity of OGG1 is impaired by Zinc deficiency. DNA Repair (Amst). **134**: 103628. https://doi.org/10.1016/j.dnarep.2024.103628

Shen Y.C., Chang Y.H., Fang C.J., Lin Y.S. 2019. Zinc supplementation in patients with cirrhosis and hepatic encephalopathy: a systematic review and meta-analysis. Nutr. J. **18**: 34. https://doi.org/10.1186/s12937-019-0461-3

Song Y., Leonard S.W., Traber M.G., Ho E. 2009. Zinc deficiency affects DNA damage, oxidative stress, antioxidant defenses, and DNA repair in rats. J. Nutr. **139**: 1626-1631. https://doi.org/10.3945/jn.109.106369

Sun Q., Zhong W., Zhang W., Li Q., Sun X., Tan X., Sun X., Dong D., Zhou Z. 2015. Zinc deficiency mediates alcohol-induced apoptotic cell death in the liver of rats through activating ER and mitochondrial cell death pathways. Am. J. Physiol. Gastrointest. Liver Physiol. **308**: G757-G766. https://doi.org/10.1152/ajpgi.00442.2014

Sun Q., Zhong W., Zhang W., Zhou Z. 2016. Defect of mitochondrial respiratory chain is a mechanism of ROS overproduction in a rat model of alcoholic liver disease: role of zinc deficiency. Am. J. Physiol. Gastrointest. Liver Physiol. **310**: G205-G214. https://doi.org/10.1152/ajpgi.00270.2015

Zhou Z., Wang L., Song Z., Saari J.T., McClain C.J., Kang Y.J. 2005. Zinc supplementation prevents alcoholic liver injury in mice through attenuation of oxidative stress. Am. J. Pathol. **166**: 1681-1690. https://doi.org/10.1016/S0002-9440(10)62478-9