AJMB
Avicenna Journal of Medical Biochemistry
Avicenna J Med Biochem. 2025;13(1):1-9. doi:10.34172/ajmb.2589
http://ajmb.umsha.ac.ir

Original Article

Assessment of Zinc Administration on Metabolic Profile, Oxidative Stress, and Gene Expression in Patients Undergoing Methadone Maintenance Treatment: A Randomized, Double-Blind, Placebo-Controlled Trial

Azam Ghaseminejad[1], Amir Ghaderi[2], Somayyeh Ghotloo[3], Hadis Aboutalebian[1], Hossein Akbari[4], Esmat Aghadavood[1], Hamid Reza Banafshe[2], Nejat Kheiripour[1*]

[1]Research Center for Biochemistry and Nutrition in Metabolic Diseases, Kashan University of Medical Sciences, Kashan, Iran
[2]Department of Addiction Studies, School of Medicine, Kashan University of Medical Sciences, Kashan, Iran
[3]Department of Medical Laboratory Sciences, School of Allied Medical Sciences, Kashan University of Medical Sciences, Kashan, Iran
[4]Social Determinants of Health Research Center, Kashan University of Medical Sciences, Kashan, Iran

Article history:
Received: January 19, 2025
Revised: April 10, 2025
Accepted: April 22, 2025
ePublished: October 31, 2025

***Corresponding author:**
Nejat Kheiripour,
Email: nejatkh.bio@gmail.com

Abstract

Background: Methadone maintenance treatment (MMT) causes important clinical problems, such as oxidative stress (OS) and inflammation. Zinc (Zn) has antioxidant and anti-inflammatory effects and regulates gene expression.

Objectives: This study investigated the effects of Zn administration on the metabolic, OS, and expression of nuclear factor erythroid 2-related factor 2 (*Nrf2*), nuclear receptor peroxisome proliferator-activated receptor γ (*PPARγ*), and *interleukin 10* genes in heroin patients undergoing MMT.

Methods: This clinical trial was conducted on 45 patients under MMT who received 30 mg/d of Zn (n = 23) or placebo (n = 22). Fasting blood samples were collected at baseline and 12 weeks after the intervention to quantify metabolic parameters, OS, and gene expression.

Results: Zn levels were significantly elevated in the intervention group compared to the placebo group. Further, consuming Zn could significantly improve high-density lipoprotein levels, insulin, homeostasis model assessment-estimated insulin resistance, and OS. Anxiety significantly decreased after 12 weeks of intervention. Finally, Zn up-regulated *Nrf2* gene expression in patients under MMT.

Conclusion: The results indicated that Zn administration could improve metabolic factors and gene expression in patients undergoing MMT

Keywords: Addiction, Opioid, PPARγ, Nrf2, Lipid profile

Please cite this article as follows: Ghaseminejad A, Ghaderi A, Ghotloo S, Aboutalebian H, Akbari H, Aghadavood E, et al. Assessment of zinc administration on metabolic profile, oxidative stress, and gene expression in patients undergoing methadone maintenance treatment: a randomized, double-blind, placebo-controlled trial. Avicenna J Med Biochem. 2025;13(1):1-9. doi:10.34172/ajmb.2589

Background

Drug use is a concerning health problem in many countries. In Iran, addiction growth is three times higher than the global prevalence, and the number of people addicted to opioids in Iran in 2019 was estimated to be around 2–4 million people (1). Methadone maintenance treatment (MMT) has been used to treat opioid dependence. Methadone is addictive, similar to other opioids. However, being on methadone is not the same as being dependent on illegal opioids (2). Tantillo et al showed that zinc (Zn) deficiency increases opioid consumption in patients undergoing total hip arthroplasty. However, Zn intake may be a simple approach to minimizing opioid use (3). Zn intake decreases oxidative stress (OS) biomarkers and inflammatory cytokines. It acts as a cofactor for enzymes, especially those that act as antioxidants (4). It has been shown that the consumption of Zn chloride decreases addiction to opioids. In addition, evidence indicates the crucial role of Zn in metabolic profile, OS, and gene expression regulation (5).

According to research, methadone therapy has been associated with oxidative stress (OS) and lipid peroxidation. Studies indicate that prolonged methadone use can lead to oxidative stress, lipid peroxidation, and

dyslipidemia, increases inflammation biomarkers, and modulates gene expression (6). Nuclear factor erythroid 2-related factor 2 (Nrf2) is a transcription factor that increases the expression of antioxidant enzymes and thus protects the cell against OS. Zn regulates *Nrf2* gene expression, while Zn deficiency suppresses its expression (7). Impaired insulin and glucose metabolism and changes in the concentration of serum lipid profiles in patients undergoing MMT may lead to long-term side effects, metabolic abnormalities, type 2 diabetes, and higher mortality. The nuclear receptor peroxisome proliferator-activated receptor γ (PPARγ) plays an important role in the regulation of insulin sensitivity, glucose, and lipid homeostasis (8). Studies have shown that Zn supplementation increases the microribonucleic acid levels of PPAR-γ and glucose transporter 1, thereby improving insulin resistance and glucose metabolism (9). Furthermore, some studies reported that MMT influences the immune system functions of opioid-dependent patients and may induce long-term systemic inflammation. The Zn deficiency resulted in the atrophy of the thymus in mice, subsequently compromising the immune function that relies on the thymus (10). Studies indicate that Zn may play a significant role in the regulation of inflammation and immune function. Interleukin-10 (IL-10) is known as an anti-inflammatory cytokine. Research conducted on pigs demonstrated that the administration of Zn supplementation resulted in a notable elevation of serum IL-10 concentration (11). Zn decreases in patients undergoing MMT, while inflammatory parameters increase in these patients, thereby decreasing the expression of antioxidant genes. Therefore, this study aims to investigate the effects of Zn administration on the metabolic profile, OS biomarkers, and *PPARγ*, *Nrf2*, and *IL-10* gene expressions in addicted patients subjected to MMT for the first time.

Materials and Methods

Trial Design and Participants

This randomized, double-blind, placebo-controlled trial study was performed on patients with opioid use disorder undergoing MMT. The present study was registered on the Iranian Registry of Clinical Trials (http://www.irct.ir, code No. IRCT20200708048050N1). To calculate the sample size, a randomized clinical trial sample size calculation formula was used, where type one (α) and type two (β) errors were 0.05 and 0.20 (power = 80%), respectively. According to a previously published trial (12), the final sample size was considered to be 30 cases under MMT aged 30–60 years in each group. They were referred to addiction-controlling clinics affiliated with Isfahan University of Medical Sciences, Isfahan, Iran, from February to May 2020. The Declaration of Helsinki guideline was followed in this study, and informed consent was given by all participants. Patients were divided into two groups to take either a 30 mg Zn supplement (n = 30) or a placebo (n = 30) for 12 weeks. No side effects were reported following the intake of Zn in patients under MMT (5). Heroin-addicted individuals treated with methadone (dose = 50 mL/day) were included in our study. Informed consent was obtained from all patients. Patients were excluded from the study if they had positive morphine test results, had taken diazepine, and had Zn-related disorders. The data related to job, education, marital status, age, age of the first use, methadone dose used (cc), duration of methadone, weight, body mass index (kg/m^2), depression, and anxiety were collected from each patient.

Measurements of Biochemical Markers, Anxiety, and Depressive Symptoms

All biochemical markers were measured at the baseline and end of the trial. Fasting blood samples (10 mL) were obtained from all participants. Enzymatic kits (Test Pars Inc., Tehran, Iran) were utilized to measure fasting blood sugar (FBS), total cholesterol (Chol), triglyceride (TG), high-density lipoprotein (HDL), and low-density lipoprotein (LDL).

The enzyme-linked immunosorbent assay kit (Monobind, California, USA) was used to determine serum insulin level. Homeostatic model assessment for insulin resistance (HOMA-IR: fasting glucose (mg/dL) X fasting insulin (mU/L) / 405) was quantified according to the standard formula (13). Zn levels were determined by the Zn Colorimetric Assay Kit (Pars Inc., Tehran, Iran). The BAI-21 questionnaire developed by Beck, Epstein, Brown, and Steer (1988) was employed to assess anxiety and depressive symptoms (14).

Measurements of Oxidative Stress Markers

The ferric reducing/antioxidant power assay was used to quantify total antioxidant capacity (TAC) based on Benzie and Strain (15). Nitric oxide (NO) was measured according to the interaction between nitrate and nitrite with Griess reagent. In addition, the glutathione (GSH) level was determined by the protocol used by Beutler et al (calorimetry assay) (16). Malondialdehyde (MDA) level was calculated by the spectrophotometric thiobarbituric acid reactive substances test (17).

Ribonucleic Acid Extraction and Real-Time Polymerase Chain Reaction

Initially, RNA was extracted according to the manufacturer's protocol (Cinnagen, Tehran, Iran). Lymphocytes were isolated using a 50% Percoll solution (Sigma-Aldrich, Dorset, UK) gradient by centrifugation for 20 minutes at 3000 rpm at 4 ˚C. Total RNA was extracted based on the acid guanidinium phenol-chloroform procedure using RNX™-plus reagent (Parstous, Tehran, Iran), according to the manufacturer's instructions. RNAs were treated with DNase I (Fermentas, Lithuania) for the elimination of any genomic DNA contamination. Thereafter, the quality and quantity of the RNA were measured by a UV spectrophotometer (OD 260/280). Next, complementary

deoxyribonucleic acid synthesis was performed from the extracted RNAs according to the manufacturer's protocol (Parstous, Iran). The real-time polymerase chain reaction (RT-PCR) was performed for *Nrf2*, *PPARγ*, and *IL-10* gene expressions. The RT-PCR was conducted on a LightCycler technology machine (Roche Diagnostics, Switzerland) with the SYBR green detection kit (Parstous, Iran). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the housekeeping gene. Forward and reverse primer sequences (5′→3′) are listed as follows:

- GAPDH, CAAATTCCATGGCACCGTCA (forward), and ATCACCCGGAGGAGAAATCG (reverse)
- Nrf2, GAATGTCTGCGCCAAAAGCTG (forward), and GAATGTCTGCGCCAAAAGCTG (reverse)
- PPARγ, AAAGGCGAGGGCGATCTTG (forward), and CCTGAAAGATGCGGATGGC (reverse)
- IL-10, CCTGCCTAACATGCTTCGAG (forward), and ACATGCGCCTTGATGTCTGG (reverse)

Relative expression levels of genes were calculated using the $2^{-\Delta\Delta ct}$ method (18).

Statistical Methods

The Kolmogorov–Smirnov test was utilized to determine the normality of data. Comparison between groups was performed using Student's t-test (anthropometric measures and gene expression) or Mann–Whitney U test (for non-normal distribution). Multiple linear regression models were employed to evaluate treatment impacts on study outcomes after adjusting for baseline values. $P<0.05$ was considered statistically significant. All statistical analyses were conducted using the Statistical Package for Social Science (SPSS) program, version 18 (SPSS Inc., USA).

Results

Overall, 7 and 8 patients were excluded from the Zn and placebo groups, respectively, because of personal reasons. Finally, 45 participants (including 23 and 22 cases in the Zn (n = 23) and placebo groups) completed the trial (Figure 1).

General Characteristics of the Study Participants

There was no significant difference in the mean of education, marital status, age, age of first use, methadone dosage, and duration of methadone use between the intervention and placebo groups at the baseline. The mean ± standard deviation (SD) of the job of the participants in the intervention group was statistically significant compared to the control group (Table 1).

Measurements of Biochemical Markers

At the beginning of the study, Zn levels were measured, and no significant difference was observed in the two groups (intervention: 82.8 ± 17.4 vs. placebo: 88.0 ± 18.2, $P=0.33$). At the end of the intervention, serum Zn level was significantly elevated in the intervention group in comparison with the placebo group. Moreover, after the 12-week intervention, the body mass index was not remarkably changed in the Zn group compared with the placebo group.

The level of lipid profile, including TG, Chol, HDL, and LDL, was also measured, and the results showed no statistically significant change in the variables, except for HDL, which significantly increased in Zn administration compared to the placebo group. Zn administration significantly reduced insulin and HOMA-IR; however,

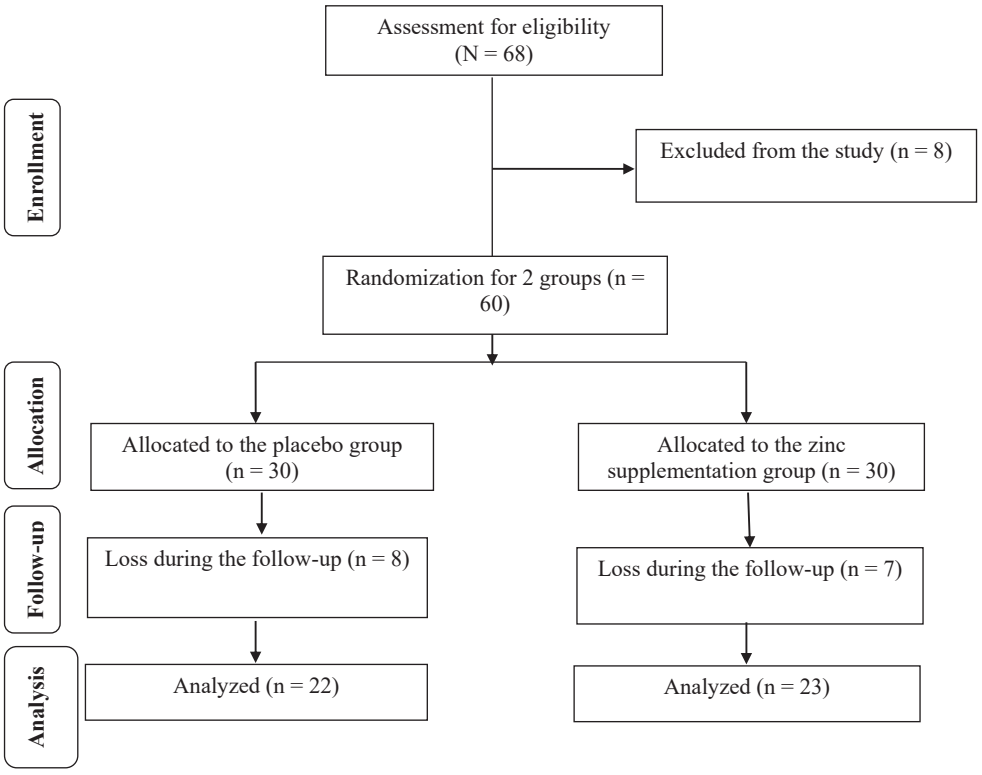

Figure 1. Summary of Patient Flow Diagram

the FBS level was not remarkably changed in the Zn group compared with the placebo group. In addition, according to the results, the depression and anxiety scores were not different between the two groups (Table 2).

OS parameters, including NO, GSH, TAC, and MDA, were assessed as well. Zn intake also caused a significant decrease in NO but an increase in GSH in comparison with the placebo group (Figure 2).

Before and after 12 weeks, the levels of biochemical markers, depression, and anxiety indicated that Zn intake significantly decreased the levels of TG, Chol, and LDL in the intervention group compared to the placebo group. Similarly, Zn significantly decreased insulin and HOMA-IR. Anxiety considerably decreased after the 12-week intervention (Table 3).

The results of OS biomarkers revealed that Zn intake

Table 1. General Characteristics of the Study Participants

Variables		Groups		*P* Value
		Zinc	Placebo	
Job (%)	Unemployed	27.3	4.3	0.02*
	Employed	9.1	0.0	
	Other	63.6	95.7	
Education (%)	Illiterate	9.1	13.1	0.90*
	Elementary	36.4	34.8	
	High school	45.5	47.8	
	University	9.1	4.3	
Marital status (%)	Single	22.7	17.4	0.57*
	Married	77.3	78.3	
	Settlement	0.0	4.3	
Age (Mean ± SD) (years)		44.0 ± 7.7	40.6 ± 6.4	0.12**
Age of the first use (years)		26.1 ± 8.0	22.4 ± 5.3	0.06
Methadone dose used (cc)		18.81 ± 6.7	19.70 ± 7.4	0.72
Duration of methadone use		4.54 ± 2.2	3.90 ± 2.1	0.34

Note. SD: Standard deviation. *Chi-square test, **Independent T-test.

Table 2. The Levels of Biochemical Markers, Depression, and Anxiety in the Two Groups After 12 Weeks of Zinc Administration Compared With the Intervention Group

Variables	Zink Group	Placebo Group	*P* value
Zinc (µg/dL)	85.0 ± 18.2	76.8 ± 15.1	0.03*
Weight (kg)	72.8 ± 13.4	70.6 ± 14.3	0.87
BMI (kg/m²)	24.0 ± 4.1	24.1 ± 4.6	0.98
TG (mg/dL)	145.6 ± 18.7	141.4 ± 39.5	0.08
Chol (mg/dL)	179.3 ± 18.0	160.1 ± 40.3	0.18
HDL (mg/dL)	50.7 ± 14.2	38.5 ± 12.7	0.04*
LDL (mg/dL)	106.6 ± 29.5	99.6 ± 28.1	0.15
FBS (mg/dL)	81.9 ± 20.1	84.6 ± 14.3	0.13
Insulin (µU/mL)	1.3 ± 0.6	1.7 ± 0.9	0.01*
HOMA-IR	0.28 ± 0.1	0.35 ± 0.1	0.01*
Depression	12.0 ± 1.2	13.0 ± 2.4	0.67
Anxiety	12.1 ± 1.5	13.0 ± 1.8	0.07

Note. BMI: Body mass index; Chol: Cholesterol; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; FBS: Fasting blood sugar; HOMA-IR: Homeostatic model assessment for insulin resistance. * $P < 0.05$

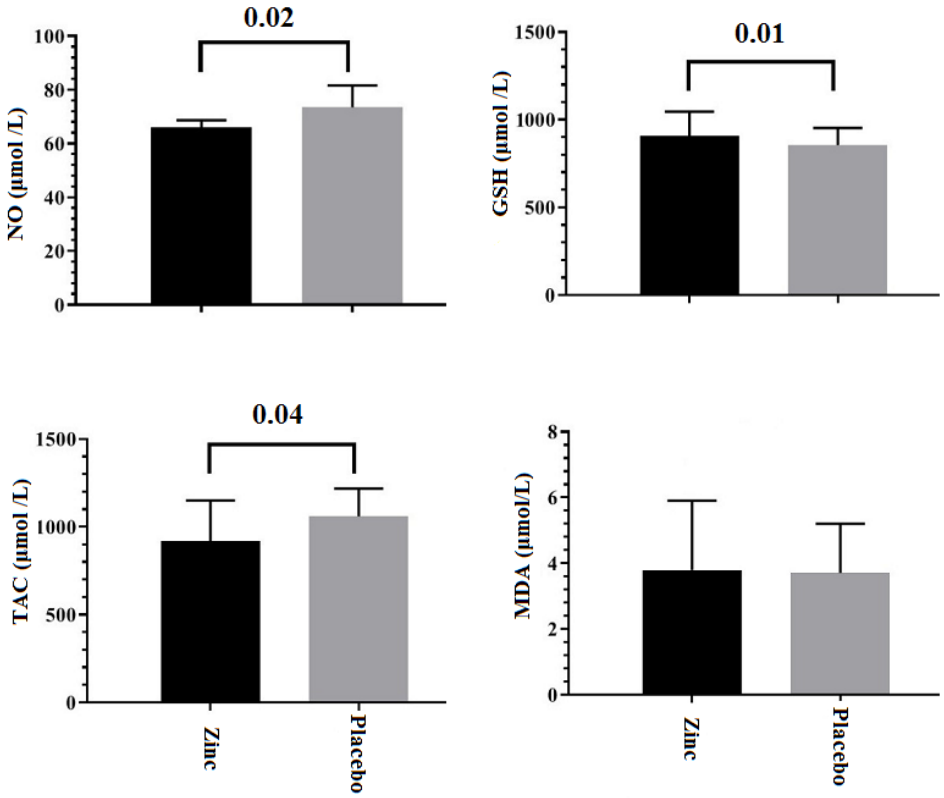

Figure 2. The Levels of Oxidative Stress Biomarkers in the Two Groups After 12 Weeks of Zinc Administration Compared with the Intervention Group. *Note*. NO: Nitric oxide; GSH: Glutathione; TAC: Total antioxidant capacity; MDA: Malondialdehyde

caused a significant increase in GSH in the intervention group before and after 12 weeks (Figure 3).

Measurement of Gene Expression Profile

RT-PCR was performed to measure the expression level of *Nrf2*, *PPARγ*, and *IL-10* genes. Zn significantly up-regulated the expression of the *Nrf2* gene when compared with the placebo in peripheral blood mononuclear cells (Figure 4). Zn administration had no significant effect on the expression of *PPARγ* and *IL-10* genes in the human peripheral blood mononuclear cells of patients under MMT (Figures 5 and 6).

Table 3. The Levels of Biochemical Markers, Depression, and Anxiety Before and After Twelve Weeks in the Intervention Group

Variables	Zinc Administration		*P* Value
	Before	After 12 Weeks	
Zinc (μg/dL)	82.8 ± 17.4	85.0 ± 18.2	0.29
Weight (g)	72.9 ± 13.3	72.81 ± 13.4	0.79
BMI (kg/m^2)	24.0 ± 4.1	24.0 ± 4.1	0.87
TG (mg/dL)	170.7 ± 85.8	145.6 ± 18.7	0.04*
Chol (mg/dL)	195.27 ± 31.90	179.3 ± 18.0	0.03*
HDL (mg/dL)	50.8 ± 11.1	50.7 ± 14.2	0.98
LDL (mg/dL)	119.7 ± 19.7	106.6 ± 29.5	0.001*
FBS (mg/dL)	79.9 ± 8.3	81.9 ± 20.1	0.65
Insulin (μU/mL)	2.1 ± 0.4	1.3 ± 0.6	0.001*
HOMA-IR	0.45 ± 0.1	0.28 ± 0.1	0.001*
Depression	15.0 ± 2.8	12.0 ± 1.2	0.09
Anxiety	17.3 ± 1.4	12.1 ± 1.5	0.001*

Note. BMI: Body mass index; Chol: Cholesterol; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; FBS: Fasting blood sugar; HOMA-IR: Homeostatic model assessment for insulin resistance; *$P<0.05$.

Discussion

To the best of our knowledge, this was the first study to evaluate the effect of 12 weeks of Zn administration on metabolic, OS profiles, and expression of *Nrf2*, *PPARγ*, and *IL-10* genes in patients undergoing MMT. After 12 weeks, the Zn group had higher levels of Zn, HDL, GSH, and expression of the *PPARγ* gene and lower levels of insulin, HOMA-IR, NO, and TAC compared to the placebo group. Furthermore, Zn administration led to a substantial reduction in state anxiety and TG, Chol, LDL, insulin, and HOMA-IR levels but a significant increase in

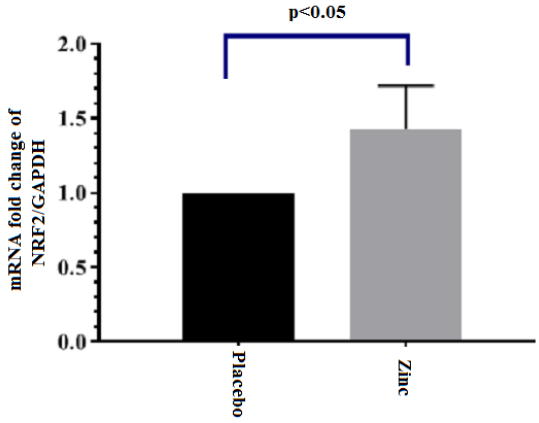

Figure 4. Measurement of the Expression Level of Nuclear Factor Erythroid 2–Related Factor 2 by Real-Time Polymerase Chain Reaction in the Two Groups, Including Intervention and Placebo Groups

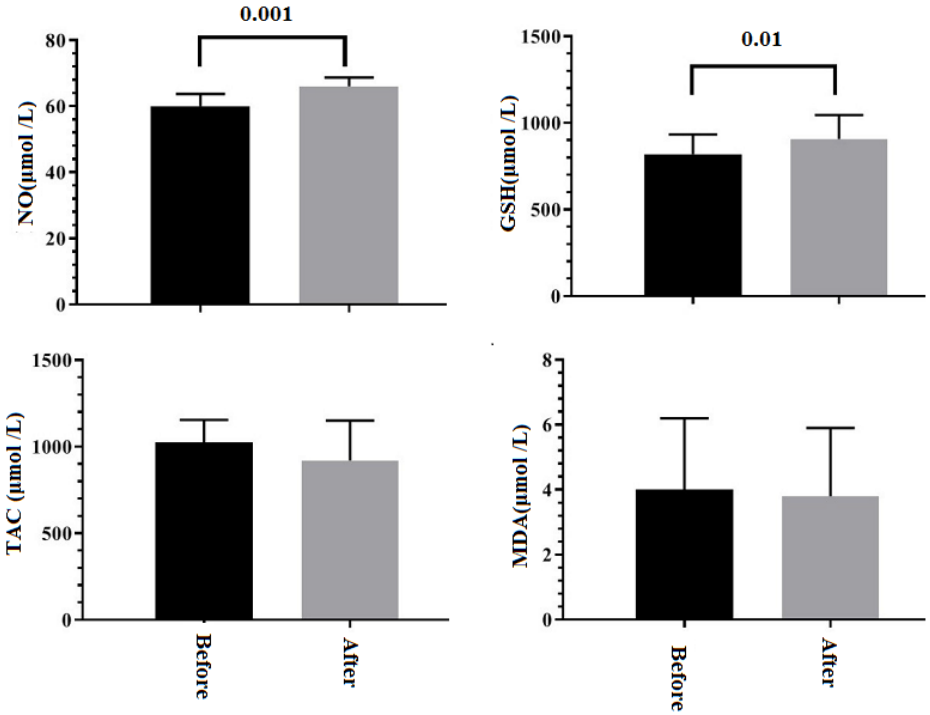

Figure 3. The Levels of Oxidative Stress Biomarkers Before and After 12 Weeks in the Intervention Group. *Note*. NO: Nitric oxide; GSH: Glutathione; TAC: Total antioxidant capacity; MDA: Malondialdehyde

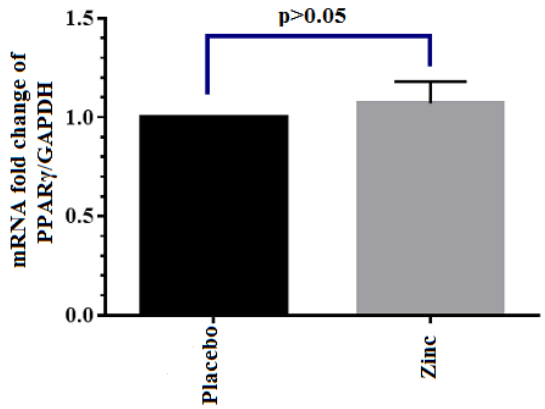

Figure 5. Measurement of the Expression Level of Peroxisome Proliferator-Activated Receptor γ by Real-Time Polymerase Chain Reaction in the Two Groups, Including Intervention and Placebo Groups

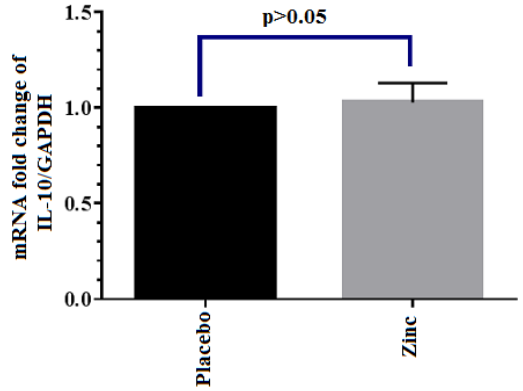

Figure 6. Measurement of the Expression Level of Interleukin 10 by Real-Time Polymerase Chain Reaction in the Two Groups, Including Intervention and Placebo Groups

NO and GSH levels.

Methadone is a synthetic opioid analgesic, similar to morphine, but with a longer duration of action and less abuse potential, and methadone medications have been approved by the Food and Drug Administration for use in treating opioid withdrawal (19). Amini et al found that Zn supplements (25 mg) combined with methadone for three months reduced opioid dependence in patients undergoing MMT (5).

Our findings demonstrated that 12 weeks of Zn intake ameliorated lipid profile in patients undergoing MMT compared with the placebo. Ghaderi et al reported a high prevalence of obesity and diabetes in patients undergoing MMT. They also concluded that these patients had high Chol concentration, hypertriglyceridemia, high blood pressure, abdominal obesity, and increased blood glucose levels (20). Vallecillo et al stated that overweight and metabolic syndrome were common in people with heroin use disorder treated with methadone (21). It was found that Zn intake had a noticeable effect on the serum concentrations of lipid metabolism. Ranasinghe et al demonstrated that Zn intake (39.3 mg/d) caused a significant reduction in LDL, Chol, and TG (22). In line with the previous study, our results revealed that there was a significant increase in the level of HDL but a decrease in TG, Chol, and LDL in the Zn group compared to the placebo group. Several possible molecular mechanisms have been suggested to be involved in the regulation of lipid metabolism by Zn intake. For example, Nakayama et al indicated that Zn inhibits hormone-sensitive lipase through a 3-kinase–protein kinase B signaling cascade by complexes dependent on the phosphoinositide (23).

Evidence demonstrates a significant association between all opioids and hypoglycemia and increased insulin concentration. Methadone treatment was associated with hypoglycemia in a study using animal models, where methadone significantly decreased blood glucose levels in a dose-dependent manner (24). Hypoglycemia caused by methadone may be due to increased insulin secretion, as well as the suppression of anti-regulatory mechanisms by decreasing glucagon and epinephrine secretion (25). Likewise, Ceriello et al observed disturbance in insulin metabolism and insulin resistance in patients undergoing MMT compared to the control group (26). Several reports showed that Zn improves glucose metabolism, regulates insulin concentration, and improves blood pressure and serum levels of LDL (27). Nygaard et al concluded that $ZnCl_2$ improves insulin secretion and increases the expression of Zn transporters ZnT3, ZnT8, and MT1A in INS-1E cells (28). Based on the results of the present study, Zn administration could significantly decrease insulin and insulin resistance after 12 weeks compared to the placebo.

OS is related to the overproduction of reactive oxygen species (ROS) and/or insufficiency of the antioxidant defense system. Frequent use of opioids increases ROS, up-regulates inflammatory factors, and contributes to opiate dependence. Zahmatkesh et al found that the administration of opioids elevates the level of ROS but decreases the function of enzymatic antioxidants, such as superoxide dismutase, catalase, and glutathione peroxidase (29). The results of the study performed by Salarian et al demonstrated an increase in OS, cell apoptosis, lipid peroxidation, and DNA damage in MMT subjects (6). The physiological levels of Zn inhibit the production of ROS. In addition, Zn acts as a cofactor for important enzymes involved in the proper functioning of the antioxidant defense system. Further, it increases the activation of antioxidant proteins and enzymes, such as glutathione and catalase (30). Interestingly, treatment with 30 mg/day Zn in the present study could significantly increase glutathione in the group receiving Zn compared to the placebo.

The Nrf2 transcription factor is the main regulator of antioxidant genes and is activated in response to OS. Nrf2 is negatively regulated by Keap1, a substrate adaptor protein for the Cullin3-containing E3-ligase complex, which targets Nrf2 for ubiquitination and degradation by the ubiquitin-proteasome system. In the conditions of the production of ROS, Nrf2 is stabilized, translocates to the nucleus, forms heterodimers with, and binds to the

antioxidant response element, leading to coordinated activation of antioxidant gene expression (31). Li et al reported that Zn up-regulated Nrf2 via the Akt-mediated prevention of Fyn nuclear translocation. Fyn is an Nrf2 negative regulator that enters into nuclei to export Nrf2 to the cytosol, where Nrf2 is degraded (32). Interestingly, treatment with 30 mg/d Zn up-regulated *Nrf2* gene expression compared to the placebo group. Studies have shown the *PPARγ* gene expression is reduced in MMT patients. *PPARγ* gene expression protects organs and tissues against fat accumulation, insulin resistance, and uncontrolled inflammatory responses. Moreover, *PPARγ* has anti-inflammatory effects and reduces the expression of inflammatory factors and the production of ROS (33,34). The expression of the *PPARγ* gene was measured in this study, and the results confirmed that there was no significant change in the expression of this gene in the group receiving Zn compared to the placebo. Many animal and human models represented that exposure to opioids reduces the production of anti-inflammatory cytokines, including IL-10, but increases the production of inflammatory cytokines (35,36). Furthermore, Zn is indicated as an adjunctive therapy for the treatment of chronic and inflammatory diseases, being able to enhance *IL-10* gene expression (37). In the present study, the level of *IL-10* gene expression in the group receiving Zn demonstrated no significant difference compared to the placebo. Long-term Zn intervention may lead to better effects in the expression of *PPARγ* and *IL-10* genes.

Amini et al showed that patients with substance abuse disorder undergoing MMT suffer from mental health conditions, including depression and anxiety. In addition, they investigated the effect of Zn intake on the probability of relapse and mental health problems in patients with opioid use disorder undergoing MMT, and their results revealed that Zn intake could decrease the probability of relapse while improving mental health problems in patients with opioid use disorder experiencing MMT (5). Our findings confirmed a decrease in depression and anxiety after 12 weeks of Zn intervention in patients under MMT, although this decrease was not significant.

Limitations of the Study

The present study had some limitations. The patients were assessed for 12 weeks. A long-term Zn intervention may lead to better effects in patients undergoing MMT. In addition, this study did not evaluate urinary Zn. Thus, it is suggested that future studies investigate the expression of genes related to inflammatory factors.

Conclusion

In summary, the results of the present study demonstrated that Zn administration for 12 weeks in patients under MMT could significantly ameliorate psychological, biochemical, insulin, insulin resistance, OS parameters, and *Nrf2* gene expression. Accordingly, Zn can be recommended as an adjunct to MMT in opioid withdrawal protocol, which may decrease methadone adverse effects.

Acknowledgments

We thank the Vice-Chancellor for Research and Technology of Kashan University of Medical Sciences and the Research Center for Biochemistry and Nutrition in Metabolic Diseases, Kashan University of Medical Sciences, Kashan, Iran.

Authors' Contribution

Conceptualization: Amir Ghaderi, Nejat Kheiripour.
Data curation: Azam Ghaseminejad, Somayyeh Ghotloo, Hadis Aboutalebian.
Formal analysis: Hossein Akbari and Hamid Reza Banafshe.
Funding acquisition: Nejat Kheiripour.
Investigation: Azam Ghaseminejad.
Methodology: Esmat Aghadavood and Nejat Kheiripour.
Project administration: Nejat Kheiripour.
Resources: Amir Ghaderi and Nejat Kheiripour.
Software: Hossein Akbari and Hamid Reza Banafshe.
Supervision: Nejat Kheiripour.
Validation: Nejat Kheiripour.
Visualization: Nejat Kheiripour.
Writing-original draft: Amir Ghaderi and Nejat Kheiripour.
Writing–review & editing: Nejat Kheiripour.

Competing Interests

The authors have no competing interests to declare that are relevant to the content of this article. They have no relevant financial or nonfinancial interests to disclose.

Ethical Approval

This study was conducted in line with the principles of the Declaration of Helsinki. The written informed consent form was signed by all the participants. Approval was granted by the Ethics Committee of Kashan University of Medical Sciences, Kashan, Iran (IR.KAUMS.MEDNT.REC.1398.134).

Funding

This article was extracted from an MSc thesis conducted with the generous support of Kashan University of Medical Sciences (project No. 98179).

References

1. Alammehrjerdi Z, Massah O, Farhoudian A, Shishehgar S, Moradi A, Dolan K. Opioid use among women on a stable methadone dose. Iran J Psychiatry Behav Sci. 2017;11(3):e9867. doi: 10.5812/ijpbs.9867.
2. Leventelis C, Veskoukis AS, Roj.as Gil AP, Papadopoulos P, Garderi M, Angeli A, et al. Methadone and buprenorphine as medication for addiction treatment diversely affect inflammation and craving depending on their doses. Pharmacy (Basel). 2025;13(2):40. doi: 10.3390/pharmacy13020040.
3. Tantillo TJ, Jagtiani MR, Silverman ER, Bitterman AD, Scuderi GR. Zinc deficiency: A cause of opioid-induced physical dependence and addiction in post-operative total hip arthroplasty patients. J Opioid Manag. 2021;17(2):145-54. doi: 10.5055/jom.2021.0607.
4. Li J, Cao D, Huang Y, Chen B, Chen Z, Wang R, et al. Zinc intakes and health outcomes: an umbrella review. Front Nutr. 2022;9:798078. doi: 10.3389/fnut.2022.798078.
5. Amini Z, Heidari Farsani E. Investigating the effect of zinc supplementation on probability of relapse and mental health in patients with opioid use disorder undergoing methadone maintenance treatment. Subst Abuse Treat Prev Policy. 2023;18(1):1. doi: 10.1186/s13011-023-00514-5.
6. Salarian A, Kadkhodaee M, Zahmatkesh M, Seifi B, Bakhshi E, Akhondzadeh S, et al. Opioid use disorder induces oxidative

stress and inflammation: the attenuating effect of methadone maintenance treatment. Iran J Psychiatry. 2018;13(1):46-54 .
7. Yu L, Liu Y, Jin Y, Liu T, Wang W, Lu X, et al. Zinc supplementation prevented type 2 diabetes-induced liver injury mediated by the Nrf2-MT antioxidative pathway. J Diabetes Res. 2021;2021:6662418. doi: 10.1155/2021/6662418.
8. Molavi N, Rasouli-Azad M, Mirzaei H, Matini AH, Banafshe HR, Valiollahzadeh M, et al. The effects of probiotic supplementation on opioid-related disorder in patients under methadone maintenance treatment programs. Int J Clin Pract. 2022;2022:1206914. doi: 10.1155/2022/1206914.
9. Ramesh V, Subbarayan K, Viswanathan S, Subramanian K. Role of nutraceuticals in the management of lifestyle diseases. In: Dhara AK, Mandal SC, eds. Role of Herbal Medicines: Management of Lifestyle Diseases. Singapore: Springer; 2023. p. 461-78. doi: 10.1007/978-981-99-7703-1_23.
10. Chan YY, Yang SN, Lin JC, Chang JL, Lin JG, Lo WY. Inflammatory response in heroin addicts undergoing methadone maintenance treatment. Psychiatry Res. 2015;226(1):230-4. doi: 10.1016/j.psychres.2014.12.053.
11. Zeng X, Song ZX, Zhou XQ, Jiang WD, Wu P, Liu Y, et al. The emerging role of zinc on alleviating inflammatory responses and enhancing immunity in grass carp (*Ctenopharyngodon idella*). Aquaculture. 2024;584:740645. doi: 10.1016/j.aquaculture.2024.740645.
12. Asghari S, Hosseinzadeh-Attar MJ, Alipoor E, Sehat M, Mohajeri-Tehrani MR. Effects of zinc supplementation on serum adiponectin concentration and glycemic control in patients with type 2 diabetes. J Trace Elem Med Biol. 2019;55:20-5. doi: 10.1016/j.jtemb.2019.05.007.
13. Pisprasert V, Ingram KH, Lopez-Davila MF, Munoz AJ, Garvey WT. Limitations in the use of indices using glucose and insulin levels to predict insulin sensitivity: impact of race and gender and superiority of the indices derived from oral glucose tolerance test in African Americans. Diabetes Care. 2013;36(4):845-53. doi: 10.2337/dc12-0840.
14. Ghaderi A, Banafshe HR, Mirhosseini N, Motmaen M, Mehrzad F, Bahmani F, et al. The effects of melatonin supplementation on mental health, metabolic and genetic profiles in patients under methadone maintenance treatment. Addict Biol. 2019;24(4):754-64. doi: 10.1111/adb.12650.
15. Hashemi G, Mirjalili M, Basiri Z, Tahamoli-Roudsari A, Kheiripour N, Shahdoust M, et al. A pilot study to evaluate the effects of oral N-acetyl cysteine on inflammatory and oxidative stress biomarkers in rheumatoid arthritis. Curr Rheumatol Rev. 2019;15(3):246-53. doi: 10.2174/1573403x14666180926100811.
16. Mehri N, Felehgari H, Larki Harchegani A, Behrooj H, Kheiripour N, Ghasemi H, et al. Hepatoprotective effect of the root extract of green tea against malathion-induced oxidative stress in rats. J Herbmed Pharmacol. 2016;5(3):116-9.
17. Devasagayam TP, Boloor KK, Ramasarma T. Methods for estimating lipid peroxidation: an analysis of merits and demerits. Indian J Biochem Biophys. 2003;40(5):300-8.
18. Kheiripour N, Plarak A, Heshmati A, Soleimani Asl S, Mehri F, Ebadollahi-Natanzi A, et al. Evaluation of the hepatoprotective effects of curcumin and nanocurcumin against paraquat-induced liver injury in rats: modulation of oxidative stress and Nrf2 pathway. J Biochem Mol Toxicol. 2021;35(5):e22739. doi: 10.1002/jbt.22739.
19. Pilarinos A, Kwa Y, Joe R, Dong H, Grant C, Fast D, et al. Methadone maintenance treatment discontinuation among young people who use opioids in Vancouver, Canada. Can J Psychiatry. 2023;68(2):89-100. doi: 10.1177/07067437221136468.
20. Ghaderi A, Rasouli-Azad M, Vahed N, Banafshe HR, Soleimani A, Omidi A, et al. Clinical and metabolic responses to crocin in patients under methadone maintenance treatment: a randomized clinical trial. Phytother Res. 2019;33(10):2714-25. doi: 10.1002/ptr.6445.
21. Vallecillo G, Robles MJ, Torrens M, Samos P, Roquer A, Martires PK, et al. Metabolic syndrome among individuals with heroin use disorders on methadone therapy: prevalence, characteristics, and related factors. Subst Abus. 2018;39(1):46-51. doi: 10.1080/08897077.2017.1363122.
22. Ranasinghe P, Wathurapatha WS, Ishara MH, Jayawardana R, Galappatthy P, Katulanda P, et al. Effects of zinc supplementation on serum lipids: a systematic review and meta-analysis. Nutr Metab (Lond). 2015;12:26. doi: 10.1186/s12986-015-0023-4.
23. Nakayama A, Hiromura M, Adachi Y, Sakurai H. Molecular mechanism of antidiabetic zinc-allixin complexes: regulations of glucose utilization and lipid metabolism. J Biol Inorg Chem. 2008;13(5):675-84. doi: 10.1007/s00775-008-0352-0.
24. Otalora Y, Inkollu S, Ursu S. Methadone induced hypoglycemia, improved on dose adjustment. J Clin Transl Endocrinol. 2020;18:100071. doi: 10.1016/j.jecr.2020.100071.
25. Tahergorabi Z, Rahmani H, Williams J, Moodi M. The effect of methadone on blood glucose, lipids and glucose-modulating hormones in methadone-dependent Wistar rats. Toxicol Res. 2020;36(3):221-6. doi: 10.1007/s43188-019-00019-z.
26. Ceriello A, Giugliano D, Passariello N, Quatraro A, Dello Russo P, Torella R, et al. Impaired glucose metabolism in heroin and methadone users. Horm Metab Res. 1987;19(9):430-3. doi: 10.1055/s-2007-1011844.
27. Cooper-Capetini V, de Vasconcelos DA, Martins AR, Hirabara SM, Donato J Jr, Carpinelli AR, et al. Zinc supplementation improves glucose homeostasis in high fat-fed mice by enhancing pancreatic β-cell function. Nutrients. 2017;9(10):1150. doi: 10.3390/nu9101150.
28. Nygaard SB, Larsen A, Knuhtsen A, Rungby J, Smidt K. Effects of zinc supplementation and zinc chelation on in vitro β-cell function in INS-1E cells. BMC Res Notes. 2014;7:84. doi: 10.1186/1756-0500-7-84.
29. Zahmatkesh M, Kadkhodaee M, Salarian A, Seifi B, Adeli S. Impact of opioids on oxidative status and related signaling pathways: an integrated view. J Opioid Manag. 2017;13(4):241-51. doi: 10.5055/jom.2017.0392.
30. Prasad AS, Bao B. Molecular mechanisms of zinc as a pro-antioxidant mediator: clinical therapeutic implications. Antioxidants (Basel). 2019;8(6):164. doi: 10.3390/antiox8060164.
31. Kaspar JW, Niture SK, Jaiswal AK. Nrf2:INrf2 (Keap1) signaling in oxidative stress. Free Radic Biol Med. 2009;47(9):1304-9. doi: 10.1016/j.freeradbiomed.2009.07.035.
32. Li B, Cui W, Tan Y, Luo P, Chen Q, Zhang C, et al. Zinc is essential for the transcription function of Nrf2 in human renal tubule cells in vitro and mouse kidney in vivo under the diabetic condition. J Cell Mol Med. 2014;18(5):895-906. doi: 10.1111/jcmm.12239.
33. Ruschke K, Fishbein L, Dietrich A, Klöting N, Tönjes A, Oberbach A, et al. Gene expression of PPARgamma and PGC-1alpha in human omental and subcutaneous adipose tissues is related to insulin resistance markers and mediates beneficial effects of physical training. Eur J Endocrinol. 2010;162(3):515-23. doi: 10.1530/eje-09-0767.
34. de Guglielmo G, Melis M, De Luca MA, Kallupi M, Li HW, Niswender K, et al. PPARγ activation attenuates opioid consumption and modulates mesolimbic dopamine transmission. Neuropsychopharmacology. 2015;40(4):927-37. doi: 10.1038/npp.2014.268.
35. Chavez-Valdez R, Kovell L, Ahlawat R, McLemore GL, Wills-Karp M, Gauda EB. Opioids and clonidine modulate cytokine production and opioid receptor expression in neonatal immune cells. J Perinatol. 2013;33(5):374-82. doi: 10.1038/jp.2012.124.
36. Hutchinson MR, Coats BD, Lewis SS, Zhang Y, Sprunger DB,

Rezvani N, et al. Proinflammatory cytokines oppose opioid-induced acute and chronic analgesia. Brain Behav Immun. 2008;22(8):1178-89. doi: 10.1016/j.bbi.2008.05.004.

37. Soares NR, de Moura MS, de Pinho FA, Silva TM, de Lima Barros SÉ, de Castro Amorim A, et al. Zinc supplementation reduces inflammation in ulcerative colitis patients by downregulating gene expression of Zn metalloproteins. PharmaNutrition. 2018;6(3):119-24. doi: 10.1016/j.phanu.2018.06.004.