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# The Utile Efficacy of Zinc and Selenium on Induction of White Adipose Tissue Browning

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## Abstract

***Background:*** We investigated the effects of zinc (Zn) and selenium (Se) (co)-supplementation (individually and in combination) on the browning process of white (WAT) to brown adipose tissue (BAT).

***Method:*** We randomly allocated obese male Wistar rats (n= 40) into five groups that were fed a high-fat diet (HFD). The control group received only HFD. The intervention groups were as follows: 1) Zn administered zinc sulfate; 2) Se administered sodium selenate; 3) Se+Zn administered combination; 4) CRD placed on a calorie restriction diet. After eight weeks of intervention, the expression of selected genes, *UCP-1*, *PGC-1α, PPAR-α, PPAR-γ, PRDM16*, and were assessed. Histological assessment of adipose and the liver tissues was also conducted using the H&E stain method.

***Results:*** The findings revealed that, expression of *UCP-1* was significantly increased in the Zn , Se , and Se+Zn groups in both WAT and BAT. It was highest in the HFD + Zn + Se ($P< 0.001$, $P= 0.029$) group. In addition, there was a decrease in *UCP-1* gene expression in the CRD group, however, it was not statistically significant. Further, the expression of *PPAR-γ* and *ZAG* gene was significantly enhanced in the HFD + Zn ($P< 0.001$, $P= 0.029$) and HFD + Zn + Se ($P< 0.001$, $P= 0.007$) groups, rather no significant difference was found in the HFD + Se ($P> 0.05$) group. Furthermore, morphological changes in tissues were consistent with the outcomes of molecular experiments.

***Conclusion:*** The findings from this study suggest that Zn and Se supplementation, particularly when combined, may effectively enhance WAT browning and thermogenesis. This could provide a promising avenue for addressing obesity through novel therapeutic strategies.

**Keywords:** Adipocytes, Adipose Tissue, Browning, Obesity, Thermogenesis.

## Introduction

Obesity is a complex, multi-stage, and preventable disorder that affects more than one-third of the world's population (1). People with obesity are at high risk of cardio-metabolic disorders, such as type 2 diabetes (T2D), hypertension, and different types of cancer, and the mortality rates are higher than those without obesity (2).

As a relatively new approach, controlling obesity can be achieved by increasing

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thermogenesis (3). *In vivo* and *in vitro* studies have indicated that activation of brown adipose tissue (BAT) results in an increase in metabolism and thermogenesis, and contributes to a reduction in obesity (3, 4)

In this study, we investigated a phenomenon known as WAT browning. During this process, WAT transforms into BAT and forms brite/beige cells. In addition to producing UCP-1, these brite cells appear to have transcription factors responsible for thermogenesis, similar to BAT. It is generally assumed that these beige cells have low thermal activity, but once activated, they can exhibit BAT characteristics (5, 6). In this regard, transcription coactivators/coregulators/factors such as PPAR-γ, PR/SET Domain 16 (PRDM16), and peroxisome proliferative activated receptor, gamma, coactivator 1 alpha (PGC-1α) primarily regulate WAT browning. In conjunction with the above-mentioned factors, UCP-1 could facilitate the browning of WAT (7). However, some studies suggest that a calorie-restricted diet (CRD), as a traditional approach to managing obesity may decrease thermogenesis and *UCP-1* gene expression (8). As a result, any factor (e.g., vitamins, minerals, antioxidants, secondary plants components, etc.) that affects one of the above-mentioned factors may lead to an increase in *UCP-1* gene expression and thermogenesis (3, 9, 10).

In animal and human studies, it has been shown that selenium (Se) supplementation could decrease lipid profiles, especially in those with low serum levels of Se (11). Furthermore, Se supplementation may increase the expression of *PPAR-γ*, *CCAAT/enhancer-binding protein alpha (C/EBPa/b)*, *PGC-1α*, and *SIRT-1* genes (12) related to the browning pathway.

On the other hand, Poiraud et al., reported that zinc (Zn) gluconate increases Peroxisome proliferator-activated receptor alpha (PPAR-α)`s transcription and function (13). Another study has suggested that zinc-a2-glycoprotein (ZAG), a soluble protein that stimulates lipid degradation in adipocytes and causes extensive fat losses, could be a potential target for obesity control (14). The study revealed an inverse association between obesity and serum ZAG levels (14). In addition, both in vitro and in vivo studies have demonstrated that ZAG activation in fatty tissues and muscles can increase *UCP-1*, *PGC-1α*, *PPAR-γ*, and *PRDM16* gene expression (15). Therefore, Zn supplementation may increase *ZAG* gene expression, which consequently may increase WAT browning.

Overall, based on the evidence, Zn and Se presumably can increase WAT browning, as they contribute to the transcription of some factors involved in thermogenesis, and this suggests a potential and promising strategy in preventing, managing, and even controlling obesity and its complications. However, to the best of our knowledge, there are very limited studies (if any), which evaluated the effects of Se and Zn (co)supplementation on thermogenesis. Therefore, we hypothesized that Ze and Se supplementation alone and combined could be effective in WAT browning via pathways, e.g., PPARs.

## Materials and Methods

### *Animals*

This study was carried out on 41 male Wistar rats (aged 21 days) and was conducted in accordance with the National Institute of Health (NIH) protocols (16). Animals were individually housed in standard metal cages. The temperature in the setting facilities was maintained at 25 ± 2 °C, while the humidity was kept at 50-55%. We maintained the rats on a 12:12 reverse light cycle (lights on at 7 A.M.). During the compatibility phase, the animals had ad libitum access to food and water for one week. Throughout the study, all necessary measures were taken to ensure a suitable environment for the animals.

### *Regimen and induction of obesity*

After a one-week adaptation period, the rats were randomly divided into high-fat diet (HFD) (n = 40) and normal diet (n = 5) groups. The normal diet group received a standard diet containing 3.6 kcal/g calories (15% lipid, 65% carbohydrate, and 20% protein), while the

high-fat group rats received a high-fat diet (HFD) with a calorie value of 5.6 kcal/gr BW. The diet provided consisted of calories per gram with a macronutrient composition of 60% fat, 20% carbohydrate, and 20% protein. The HFD was prepared and assembled by the Royan Biotechnology Institute of Isfahan (Isfahan, Iran).

We considered the obesity model successfully induced, when the HDF group gained approximately 25% more weight than the normal diet group (after 20 weeks). Then, at the end of the 20$^{th}$ week, the rats were randomly divided into four groups: 1) Ze which received zinc sulfate (15 mg/kg body weight); 2)Se which received selenium in the form of sodium selenate (0.5 mg/kg body weight); 3) Zn + Se which received zinc (15 mg/kg body weight) with selenium (0.5 mg/kg body weight) and 4) HFD without any supplementation. For all groups, the interventions lasted for 8 weeks.

***Sample collection***

Upon completing the intervention (8 weeks), animals were anesthetized with xylazine and ketamine hydrochloride, which was followed by cardiac puncture for blood collection. A low-speed centrifugal separation of the plasma at 2,500 × g for 15 minutes at 4 °C was immediately performed, and the plasma was stored at -80 °C. After removing the inguinal WAT, one piece was washed with phosphate-buffered saline (PBS), frozen in liquid nitrogen, and stored at -80 °C for further analysis. The other part (three rats in each group) was placed in formalin buffer at room temperature for 7 days for histological examination. In our previous study, we reported the methods for measuring weight, feed intake, antioxidant profile, and markers of lipid peroxidation and inflammation (17).

***Histological assessment***

A portion of tissue from three randomly selected rats in each group was placed in 50 ml Falcon tubes containing 10% formalin for 7 days at room temperature, with the solution being changed every 2 days. Subsequently, the tissues were sectioned horizontally and dehydrated using alcohol solutions of varying concentrations. They were then embedded in molten paraffin. After drying, the resulting paraffin molds were cut with a thickness of 5 µm using a microtome machine (DS4055, Did Sabz Company), and the tissue was fixed on the slide. In the next step, the slides were stained using the hematoxylin and eosin (H&E) method. The stained cells were collected after one day of exposure at ambient temperature and examined by an expert person who was blinded to the intervention groups with a Nikon light microscope with 40× magnifications.

***Biochemical analysis***

***RNA extraction***

After weighing the frozen lipid specimen, 50-100 mg of sample were separated and homogenized in one ml of Trizol (BIO BASIC, Canada, Cat. #BS409A). The samples were then vortexed for 5-10 seconds and incubated at room temperature for 5 minutes. 200 µl of chloroform was added to the specimens, followed by 2-3 minutes of incubation at room temperature. Afterward, the samples were centrifuged for 15 minutes at 2-8 °C at 12,800 × g. In a 1.5 ml microtube, we mixed the solution phase with an equal amount of isopropanol solution and then incubated it for 10 minutes at 15-30 °C. Following this, the samples was centrifuged at 4 °C for 10 minutes at 12,800 × g. To wash RNA sedimentation, 1 ml of ethanol 75% was added to the specimen. It was then vortexed and centrifuged at 4 °C for 5 minutes at 6,300 × g**.** The RNHFDA pellet was dried, then dissolved in 20 uL of DEPC water and spun for 10 minutes. A NanoDrop Spectrophotometer (NanoDrop One/Onec, Thermo Scientific) was used to measure the RNA concentration according to the manufacturer's instructions. Since cDNA has higher stability and quality than RNA, cDNA synthesis was performed using a TaKaRa synthetic kit (PrimeScript™ RT reagent Kit, Cat. #RR037A).

### *Quantitative PCR*

The specific primer sequences for targeted genes such as *UCP-1* (Gene ID: 24860), *PGC-1α* (Gene ID: 83516), *PPAR-α* (Gene ID: 25747), *PPAR-γ* (Gene ID: 25664), *PRDM16* (Gene ID: 100366024), and *ZAG* (Gene ID: 25294) were designed using the NCBI Primer Bank. The final concentration was 0.5 µmol. Specific primer sequences are listed in Table 1.

**Table 1.** The primer sequence of examined genes.

| Gene | Forward | Reverse |
|---|---|---|
| *UCP-1* | CGTGGCGGTATTCATTGG | CTATAACTCTGTAAGCATTGTAGG |
| *PPAR- α* | GGTCATACTCGCAGGAAAG | GCAGCAGTGGAAGAATCG |
| *PPAR- γ* | TGGAGCCTAAGTTTGAGTTTG | CAGCAGGTTGTCTTGGATG |
| *PRDM16* | ACGACCACCTCTGCTACCTC | GGGCTCCTATTTTTGACCTTCT |
| *PGC-1 α* | AAGTAGACAAGACCAGTGAAC | GCAAGAAGGCGACACATC |
| *ZAG* | ACGAGGACAGTACAGGGTCTC | TCCAGGCTGGGATTTCCTTG |
| *β-actin* | CGGTCAGGTCATCACTATCGG | ATGCCACAGGATTCCATACCCA |

### *Data analysis*

All the data were analyzed using IBM SPSS software (IBM SPSS Statistics 20, Armonk, NY, USA). Before data analysis, the distribution of variables was assessed using statistical tests such as the Kolmogorov-Smirnov test and histogram diagrams. Parametric analysis was employed for normally distributed variables, whereas non-parametric analysis was utilized for non-normally distributed variables, which were demonstrated as mean and standard deviation (SD). One-way ANOVA and Tukey's post hoc tests were used to compare the means of variables between different groups. The mean of the variables studied between the two groups was compared using an independent t-test. To compare results among studied groups, we used analysis of covariance (ANCOVA) to obtain baseline adjustment. To evaluate the mean weight at different times in each group, we used two-way ANOVA and Tukey's HSD post hoc test. Diagrams were created using GraphPad Prism (GraphPad Software, Inc., San Diego, CA, version 8). Statistical significance was defined as $P< 0.05$.

## Results

### *Induction of obesity by high-fat diet (HFD)*

Throughout the study, no animals were lost, and all rats underwent the interventions as planned. The rats included in this study were all in their fourth week of life. Following a week of acclimatization, the use of HFD for 20 weeks resulted in an increase in weight of 473.18 ± 35.49 grams compared to the same group on a regular standard diet, which gained 393.25 ± 22.3 grams ($P<0.05$). Given that the subjects' body weight increased by approximately 25%, we established an obesity model to commence supplementation. We used the HFD group as the control group and compared it to intervention groups supplemented with Se , Zn , Se+Zn , and the CRD group.

### *Weight and dietary intake*

Weight changes and feed intake of the intervention groups have been reported elsewhere (17). According to the mentioned study, the greatest weight loss and decrease in feed intake compared to the control group were observed in the group that received the

combination of zinc and selenium supplements, the Zn+Se group (P< 0.05) (17).

***Se group***

A significant increase in *UCP-1* gene expression was observed in BAT and WAT of the Se group when compared to the control group (P< 0.001, P= 0.029). Additionally, there was a statistically significant increase in the expression of *PPAR-α* gene compared to the control group in both BAT and WAT (P= 0.002, P< 0.001). However, *PPAR-γ* mRNA levels in WAT and BAT of the Se group were not significantly elevated (P= 0.05). *PRDM16* mRNA levels in WAT did not significantly increase (P> 0.05), whereas they were significantly higher in BAT (P= 0.028). Furthermore, both WAT and BAT exhibited a significant increase in *PGC-1α* gene expression (P= 0.028, P= 0.004). In addition, there was a nonsignificant increase in ZAG gene expression in both WAT and BAT (P>0.05).

***Zn group***

A significant increase in *UCP-1* gene expression was observed in both WAT and BAT of the Zn group compared to the control group (P= 0.001, P= 0.028). The expression of *PPAR-α* gene was also significantly increased in both WAT and BAT (P= 0.001, P<0.001). Additionally, both WAT and BAT expressed *PPAR-γ* gene (P= 0.012, P<0.001), *PRDM16* gene (P= 0.0013, P=0.012), and *PGC-1α* gene (P=0.047, P=0.013) at substantially higher levels. Despite the fact that Zn supplementation did not significantly increase *ZAG* mRNA in WAT (P= 0.053), the results were significant in BAT (P= 0.029).

***Zn+Se group***

We detected a significant increase in the expression of all genes associated with WAT browning and thermogenesis in the group supplemented with both Zn+ Se, possibly due to their synergistic effects. In this group, both WAT and BAT showed significant increases in the level of *UCP-1* mRNA (P< 0.001, P= 0.002), *PPAR-α* gene (P< 0.001, P< 0.001), *PPAR-γ* gene (P= 0.004, P< 0.001), *PRDM16* gene (P= 0.001, P= 0.009), and *PGC-1α* gene (P= 0.001, P< 0.001). In contrast to the groups supplemented with Zn and Se alone, this group showed significant increases in *ZAG* gene expression in both WAT and BAT (P= 0.004, P= 0.007).

***Calorie-restricted diet (CRD) group***

Both WAT and BAT genes in the CRD group displayed decreased *UCP-1* gene expression and mRNA levels, which were not significant (P= 0.977, P= 0.924). When comparing the CRD group with other intervention groups, all the supplemented groups showed a significant increase in *UCP-1* gene expression in both WAT and BAT (P<0.001).

In the CRD group, *PPAR-α* gene expression was significantly increased in both WAT and BAT compared to the control group (P= 0.004, P< 0.001) but was not significant when compared to the intervention groups (P> 0.05).

The level of *PPAR-γ* mRNA was elevated in the CRD group, although this increase did not reach statistical significance compared to the control group. In contrast, a significant difference in *PPAR-γ* mRNA level was observed between the CRD group and the Zn and Zn+Se groups only in the WAT (P= 0.004, P= 0.001).

In both WAT and BAT, the *PRDM16* gene was not significantly altered in the CRD group compared with the control group (P>0.05). However, compared with all three intervention groups, the *PRDM16* gene was significantly altered in the BAT of the CRD group (P< 0.05), whereas in WAT, it was significant only when compared with the Zn+Se group (P= 0.012).

The level of *PGC-1α* mRNA was not significantly increased in both BAT and WAT of the CRD group compared to the control group (P>0.05). However, WAT and BAT of the supplemented groups showed significant increases compared to the CRD group (P< 0.05).

We detected an increase in *ZAG* gene expression in the CRD group, but the difference was not significant compared to the control group. While *ZAG* gene expression increased significantly in the Zn and Se groups compared

to the CRD group, *ZAG* mRNA levels did not significantly increase in these two supplemented groups (P> 0.05).

***Histological and morphological changes***

No evidence of browning was observed in WAT histology in rats of the HFD group. When assessing BAT in the HFD group, reverse conversion of BAT to WAT was seen in some areas. The results of the present study indicate that supplementation with Zn and Se leads to the creation of adipocytes similar to brown adipocytes, or in other words, beige adipose tissue containing smaller and denser adipocytes in the WAT of rats in all three groups supplemented with Zn , Se , and Zn+Se compared to the same tissue in the HFD group without supplementation.

The direction of BAT changes in the rats of Zn, Se , and Zn+Se groups was towards a higher density of adipocytes, smaller fat drops, and darker color of BAT compared to rats consuming HFD and without supplementation. In addition, more capillaries and a higher density of adipocytes compared to the BAT of HFD group rats were among the other characteristics of the intervention groups.

In WAT, the adipocytes of the group receiving the combination of both supplements had a smaller size, higher density, and more blood vessels than the groups consuming Zn and Se alone. Also, BAT in the Zn+Se group had a darker brown appearance than BAT in the Zn and Se groups (Fig. 3).

In the CRD group, smaller and denser adipocytes were observed in WAT than in the HFD group. Few white adipocytes were found near the BAT of CRD group rats, but a smaller number was observed compared to the adipocytes in the BAT of the HFD group. The number of changes in the CRD group toward the conversion of white adipose tissue to brown and the activation of BAT were also insignificant compared to the groups receiving Zn and Se supplements along with HFD.

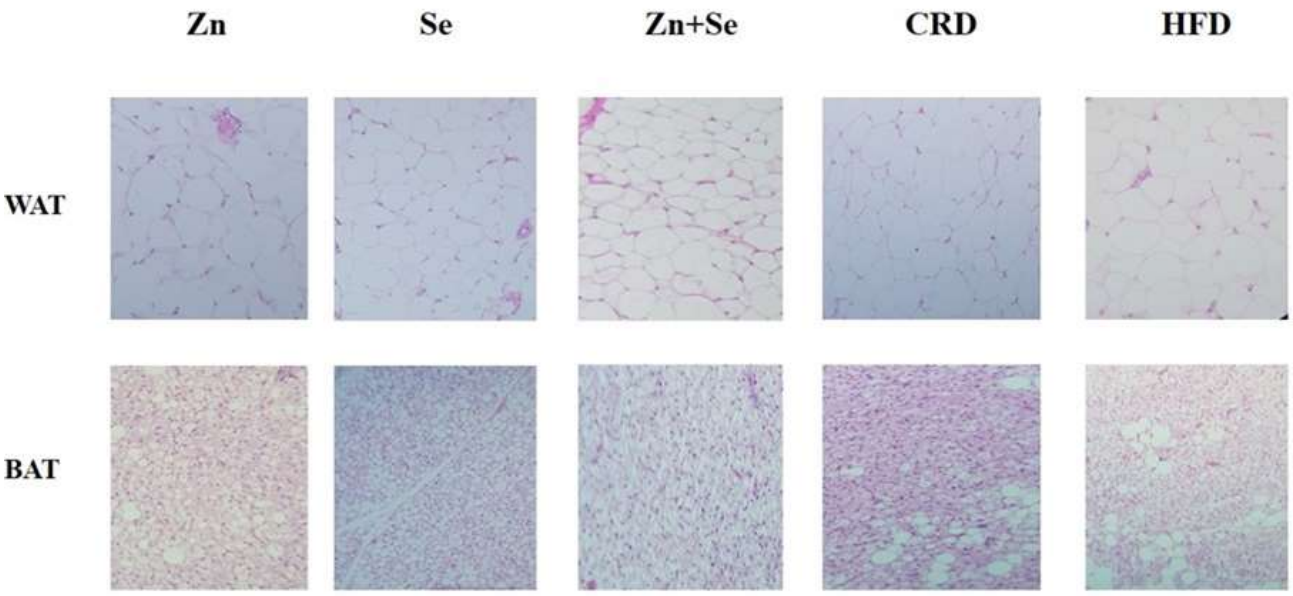


**Fig. 3.** Representative histological sections of adipose tissues from experimental groups. Sections of white and brown adipose tissue stained by H&E method.
WAT: white adipose tissue; BAT: brown adipose tissue; HFD: high fat diet; CRD: calorie restriction diet; Zn: zinc; Se: selenium.

***The histological results of liver tissue in the intervention groups***

After 28 weeks of consuming a high-fat diet, rats in the HFD group exhibited larger livers with a darker color and harder texture compared to the intervention groups that received supplementation. Accumulation of fat droplets was seen sporadically in the liver

tissue of the HFD group. In contrast, in the groups receiving HFD with supplements, fat accumulation in liver cells was decreased compared to the control group (HFD). The highest reduction rate was observed in rats receiving the Zn+Se group. In this group, the liver had a texture similar to normal and had a smooth and red surface.

In the rats of the CRD group, similarly to the groups under supplementation, there was a decrease in the amount of fat accumulation in the liver tissue compared to the HFD group. However, this reduction rate was lower for the Zn, Se, and Zn+Se groups (Fig. 4).

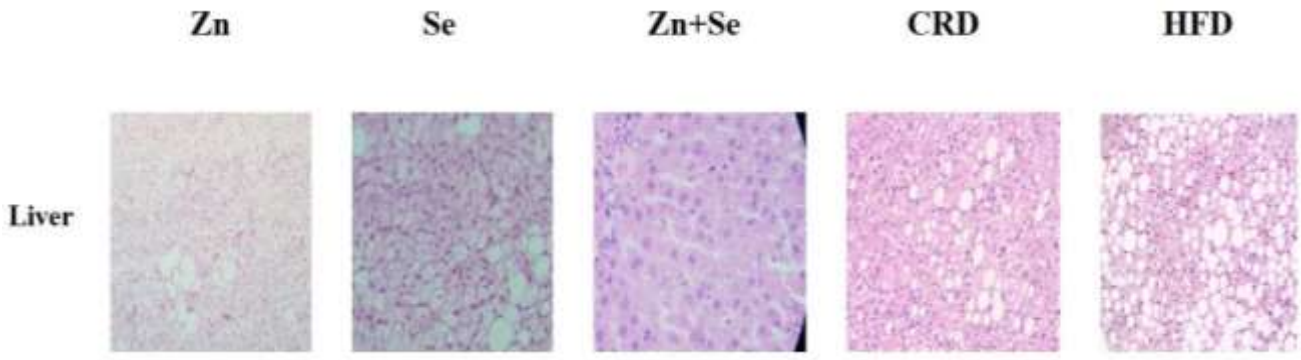


**Fig. 4.** Representative histological sections of liver from experimental groups. Liver sections stained by H&E method. WAT: white adipose tissue; BAT: brown adipose tissue; HFD: high fat diet; CRD: calorie restriction diet; Zn: zinc, Se: selenium.

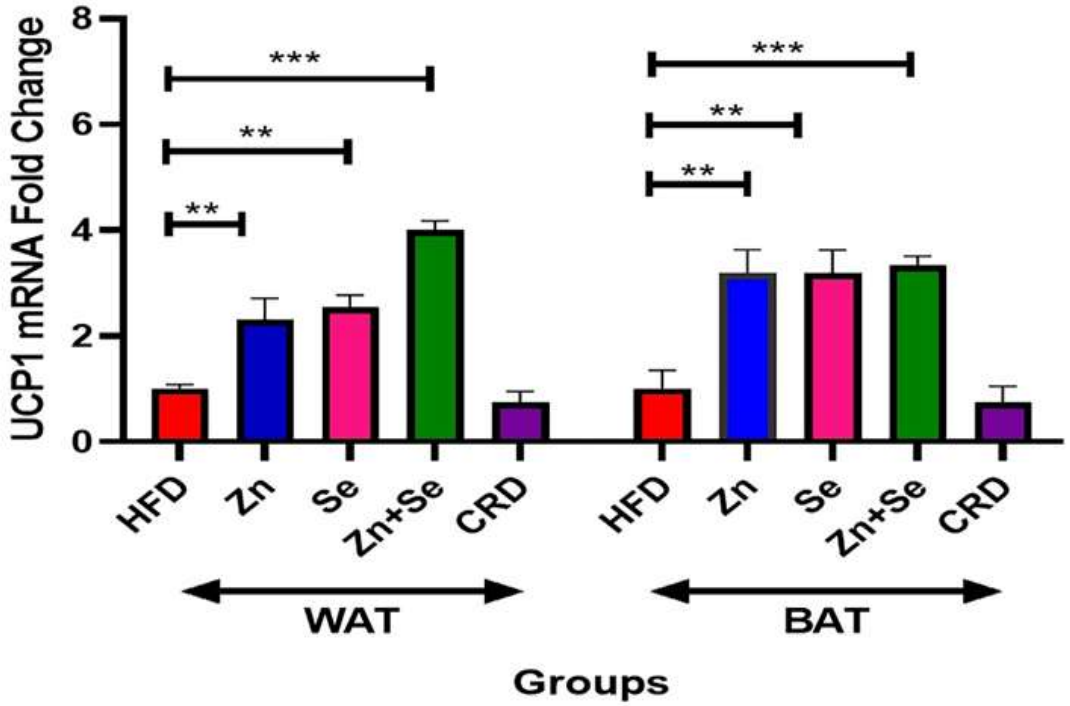


**Fig. 1.** The expression of *UCP-1* in white and brown adipose tissue in intervention and control groups. WAT: white adipose tissue; BAT: brown adipose tissue; HFD: high fat diet; CRD: calorie restriction diet; Zn: zinc; Se: selenium.

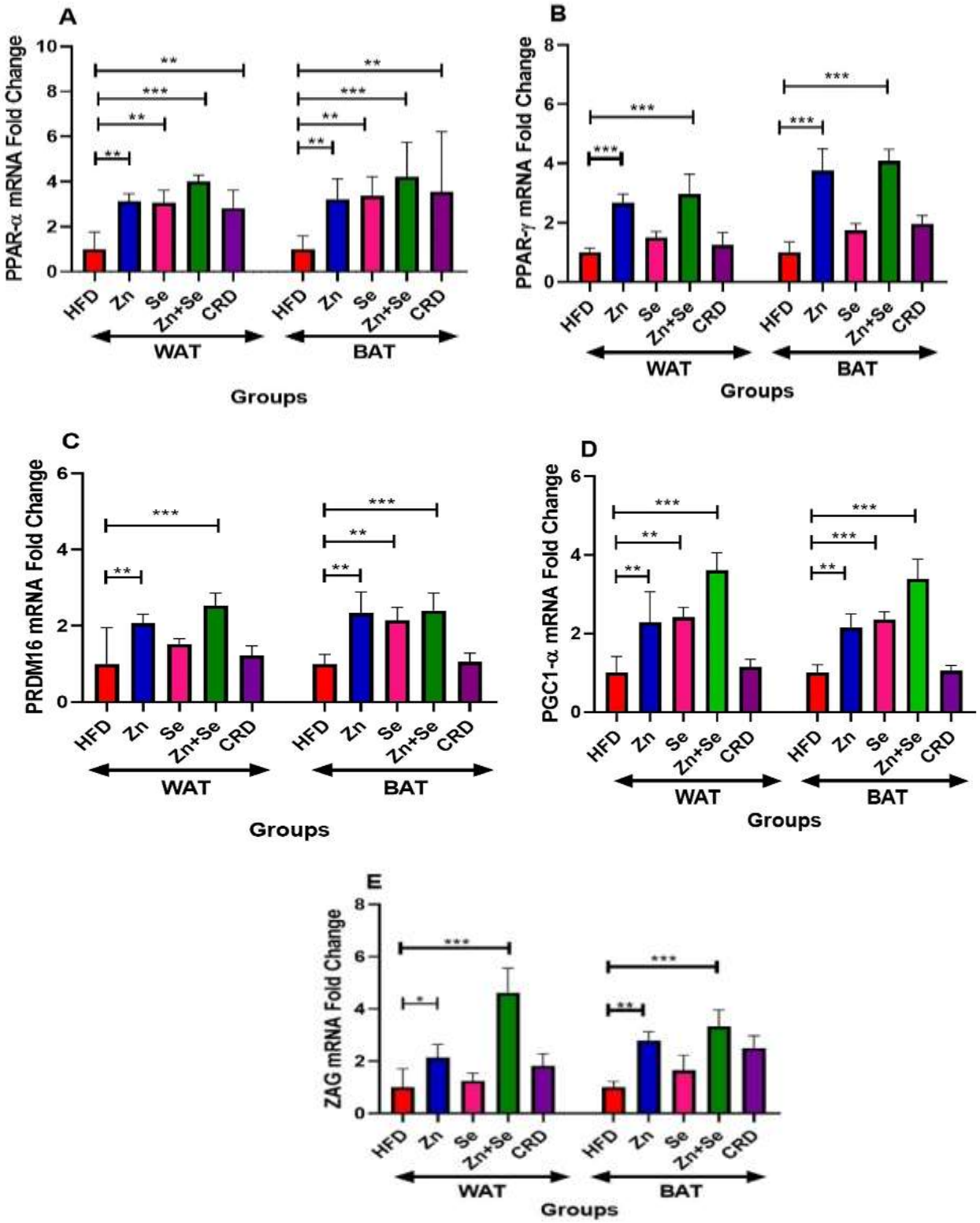


**Fig. 2.** The expression of PPARα, PPARγ, PGC-1α, PRDM16, and ZAG in white and brown adipose tissue in intervention and control groups. WAT: white adipose tissue; BAT: brown adipose tissue; HFD: high fat diet; CRD: calorie restriction diet; Zn: zinc; Se: selenium.

## Discussion

Our study findings indicate that supplementation with selenium (Se) increases *UCP-1* gene expression in both white adipose tissue (WAT) and brown adipose tissue (BAT). However, the effect is notably more

pronounced in WAT, possibly due to the higher presence of free fatty acids acting as ligands. Increased levels of UCP-1 could facilitate WAT browning, which results in the production of more brite/beige cells with a higher thermal capacity. Jedrychowski et al. (18) evaluated the effects of Se on the expression of *UCP-1* gene, and their findings were consistent with our results. In addition, according to another study (19), Se supplementation enhanced the expression of *PGC-1α* gene in both BAT and WAT.

Moreover, PGC-1α is responsible for the production of liver selenoproteins, which may explain why a decrease in *PGC-1α* gene expression is associated with Se deficiency (20). *PPAR-α* is another gene essential for the expression of *UCP-1*gene. In line with previous studies, we found an increase in the expression of *PPAR-α* gene after supplementation with Se (21). Although *PPAR-γ* gene expression increased in both types of adipose tissue (WAT and BAT) in the Se supplemented group compared to the control, the increase was not statistically significant. Previous studies have shown different and debatable results, e.g., some studies concluded that Se supplementation might decrease *PPAR-γ* gene expression (22), while a study indicates that Se supplementation may enhance *PPAR-γ* gene expression (23). The inconsistent results may be attributed to different chemical structures and dosages of Se supplement, target tissues, and timing of interventions. Upon supplementation with Se, *PRDM16* gene expression was increased in BAT, but not in WAT. This might be attributed to BAT having a greater number of PGC-1α-PPAR complexes, which may promote *PRDM16* gene expression (7, 24). Furthermore, our study found no significant improvement in ZAG gene expression in the Se-supplemented group, and there are very limited, if any, published studies that have evaluated the effects of Se and Zn on *ZAG* gene expression.

In the Zn supplemented group, there was an elevation in *UCP-1* gene expression, consistent with findings reported by Lou et al.(25). Furthermore, we found increased *PGC-1α* gene expression in the Zn-supplemented group; nevertheless, there is a lack of supporting literature on this outcome. In our study, *PPAR-α* gene expression was increased in the Zn-supplemented group, in line with findings reported by Kang et al.(26), which indicated that Zn supplementation increases PPAR-α levels in hepatocytes and DNA binding. Regarding *PPAR-γ*, we observed an increase in its expression in WAT; however, there are very limited studies that have directly assessed the effect of Zn on PPAR-γ in obesity. However, Heidarzadeh et al. (27) have shown that PPAR-γ levels increase following Zn supplementation in pregnant women with gestational diabetes.

Furthermore, Zn deficiency is known to decrease PPAR-γ gene expression and function (28). Additionally, we observed an increase in *ZAG* gene expression in WAT and BAT in the Zn supplemented group. Zn plays a critical role in ZAG production, and a deficiency in Zn may result in decreased *ZAG* gene expression.

This study is among the pioneering evaluations of the synergistic effects of Zn and Se in controlling obesity. There was a significant increase in the expression of all genes involved in thermogenesis and WAT browning in the Zn+Se supplemented group, including *UCP-1*, *PPAR-γ, PGC-1α, PPAR-α, PRDM16*, and *ZAG* genes. Furthermore, the Zn + Se group had significantly higher gene expression than the Zn and Se groups individually, possibly due to the synergistic effects.

The expression of these genes was also compared between the CRD group and the other intervention groups that received HFD. According to our results, there was no significant difference between the CRD and control groups in terms of *UCP-1* gene expression. This finding has been confirmed by another study, providing additional support and validation to the results observed in our research (29). However, in the CRD group, *UCP-1* gene expression decreased by 25% and 27% in WAT and BAT, respectively.

Nevertheless, this decrease was not statistically significant. Furthermore, some studies have also demonstrated a reduction in *UCP-1* gene expression in CRD groups (8, 30), suggesting that a longer intervention period would substantially reduce *UCP-1* gene expression. Accordingly, it appears that in a situation of restricted energy intake, the body decreases the expression of *UCP-1* gene as well as thermogenesis, supporting our hypothesis that *UCP-1* gene expression is lower in the CRD group. We were unable to demonstrate a significant effect on *PGC-1α, PPAR-γ*, or *PRDM16* genes in the CRD group; however, as shown in other studies (31), PPAR-γ expression would have increased with a longer study duration. *PPAR-α* gene expression was increased in the CRD group, as reported in Soltis et al.'s study (32), but this difference was not statistically significant. *ZAG* gene did not significantly increase in the CRD group either.

The results of the morphological studies are in line with the molecular findings obtained from the present study, which showed that the expression of thermogenic genes in the WAT of rats supplemented with Zn and Se increased significantly. Examination of groin WAT in rats of all three intervention groups, along with consumption of a high-fat diet, showed that the intake of Zn and Se led to the creation of smaller and denser adipocytes with more limited lipid reserves, which exhibited the same phenotype as brown adipocytes and demonstrated conversion of WAT to inguinal adipose tissue. This evidence was more strongly observed in the group receiving the combination of two supplements.

In addition, the histological examination of WAT and BAT of the CRD group confirmed the findings of molecular tests and the results of gene expression studies. In this way, the white adipocytes in rats receiving CRD had a smaller size than these adipocytes in rats consuming HFD, and this difference suggests a lower triglyceride content following caloric restriction. Regarding BAT changes in the interscapular area, histological studies showed that in both CRD and HFD-consuming groups, in the vicinity of dense brown adipocytes, there were white adipocytes in the form of bubbles with larger fat droplets, which confirmed the weak effects of CRD on activation of BAT. Based on the results, adding Zn and Se to the high-fat diet led to the creation of smaller and denser white adipocytes and a change in color from white to creamy and beige. In these groups, cells with the phenotype of brown adipocytes, which were smaller and containing more vessels, were visible. Also, in the brown fat tissue, the rats receiving Zn and Se alone and in combination had fewer white adipocytes than the HFD control group. In contrast, larger adipocytes with more fat content were observed in the vicinity of dense brown adipocytes of BAT in the group receiving HFD; this was observed in lower amount in the supplement-consuming groups. Overall, the results of the histological examinations in both the HFD and CRD groups were consistent with the findings related to the expression of thermogenic genes.

On the other hand, long-term consumption of a high-fat diet led to the induction of conditions like fatty liver in HFD group rats. The accumulation of fat droplets in large sizes was evident in the liver tissue of the HFD group. In contrast, administration of Zn and Se supplements visibly reduced the accumulation and size of fat droplets in the liver tissue of these groups. In the group receiving the combination of two supplements, the reduction in the amount of fat accumulation was significantly higher than in the other two groups, resulting in liver tissue had a similar appearance to the normal liver. In the CRD group, the liver had a lighter color, and fewer fat droplets were observed than in the HFD group. However, the amount of fat accumulation in this group was higher compared to the groups receiving supplements. The results of Musavi et al.'s study, consistent with the results of the present study, showed that 20 weeks of consuming a high-fat diet (80% of calories from fat) causes NAFLD in Sprague Dawley rats (33). In this study, the combined intake of Zn and Se supplements after the induction of the NAFLD model

caused a decrease in fat accumulation in the intervention groups compared to the control group.

Our study had some strengths and limitations. To the best of our knowledge, this is the first study that has evaluated the effects of Zn and Se supplementation and their co-supplementation on reversing obesity through thermogenesis and WAT browning, however, there is still much to be explored in this field. In addition, the simultaneous study of several pathways, including the expression of thermogenic genes in obese rats, is another strength of this research.

Due to budget limitations, we were unable to examine all the factors that influence WAT browning. As potential methods, we suggest measuring the protein levels produced by different genes during this process using Western blots and measuring AMPK, P38-MAPK, and SIRT1 levels because they considerably impact the expression of *UCP-1* and *PGC-1α* genes. Additionally, measuring the basal metabolic rate through exercise or cold tolerance tests can help provide a more accurate interpretation of the results, which represents one of the limitations of the present study.

In conclusion, we demonstrated that selenium and zinc supplementation resulted in weight loss following HFD-induced obesity due to a significant increase in the expression of the *UCP1* gene (a key gene involved in thermogenesis and browning) and some genes affecting its induction and activation, including *PPARα, PPARγ, PGC-1α, PRDM16*, and *ZAG* in WAT and BAT across different groups compared to the control group. Caloric restriction did not significantly affect the expression of most of the genes involved in the thermogenesis process. The combination of Zn and Se reduced obesity more effectively than either element alone. Further research is required to determine the exact mechanisms involved.

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## Ethics approval

The research ethics code number (IR.GOUMS.REC.1397.577) was obtained from the Ethics Committee of IRAN University of Medical Sciences**.**

## Competing Interest

The authors declare no conflict of interest.

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## Author Contributions

Conceptualization, M.H**,**.; methodology, M.H and M.V.; validation, J.H.; investigation, V.M. and M.H; resources, M.V and M.KH.; data curation, E.A. and A.J.; writing—original draft preparation, A.J. and E.A.; writing—review and editing, M.H and F.V; visualization, F.V.**,** and M.H; supervision, M.H.; project administration, M.H.**;** All authors have read and agreed to the published version of the manuscript..

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