The effects of zinc sulfate supplementation on serum copeptin, c-reactive protein and metabolic markers in zinc-deficient diabetic patients on hemodialysis: a randomized, double-blind, placebo-controlled trial

Article (Accepted Version)

The effects of zinc sulfate supplements on metabolic markers in zinc-deficient diabetic patients on hemodialysis: A randomized, double-blind, placebo-controlled trial

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Abstract:

Objective: We aimed to investigate the association between zinc (Zn) supplementation and serum levels of copeptin and high sensitive C-reactive protein (hs-CRP), glycemic control and renal function in zinc-deficient diabetic hemodialysis patients (DHPs).

Methods: This randomized, double-blind, placebo-controlled trial (RCT) was conducted on 46 DHP with Zn-deficiency. The Zn supplement group (n = 21) received a 220 mg/day Zn sulfate capsule (containing 50 mg Zn), and the control group (n = 25) received a placebo capsule (220 mg corn starch), for 8 weeks. Fasting, predialysis blood samples were taken at baseline and after 8 weeks to assess fasting blood glucose (FBG), serum insulin, copeptin, high sensitivity C-reactive protein (hs-CRP), Blood Urea Nitrogen (BUN), creatinine (*Cr*) concentrations and, homoeostatic model assessment (HOMA-IR) and quantitative insulin sensitivity check index (QUICKI).

Results: Compared to controls, serum copeptin ($P < 0.001$), hs-CRP ($P < 0.001$), BUN ($P < 0.001$), Cr ($P < 0.001$), Zn ($P < 0.001$), FBG ($P < 0.001$) levels, BMI ($P < 0.001$) and body weight ($P < 0.001$) were significantly affected following ZnSO4 supplementation for 8 weeks. In contrast, QUICKI ($P = 0.57$), HOMA-IR ($P = 0.60$) and serum insulin ($P = 0.55$) were not affected following Zn supplementation in comparison with patients receiving placebo.

Conclusion: Zn sulfate supplementation appears to have favorable effects on serum copeptin and hs-CRP, FBG and renal function in Zn-deficient DHPs.

Keywords: Zinc, Copeptin, Diabetic nephropathy, Glycemic indices, Inflammation.

Introduction

Diabetic kidney disease (DKD), often develops in diabetic patients, and have different aetiologies, but is particularly due to hypertensive nephrosclerosis (1), which is the single most common cause of the end-stage renal disease (ESRD) in the Western populations (2). Mortality is high in patients with ESRD for various reasons, including systemic inflammation and protein energy malnutrition (PEM); hemodialysis patients (HP) are at risk for deficiencies in macronutrients and micronutrients, which can induce oxidative stress and inflammation (3). Levels of inflammatory markers such as C-reactive protein (CRP), tumor necrosis factor alpha (TNF-α), interleukins 1,6 and 8 are often increased in HP (4).

Zinc (Zn) is an essential trace element, first noted in the 1960's by Prasad et al. and it is the second most abundant trace metal after iron in the human body (5). Zn may reduce chronic inflammation by its effects on serum inflammatory cytokines and oxidative stress due to its role as a cofactor for enzymes and, participating in synthesis of antioxidant enzymes, lipid, protein and, carbohydrate metabolism (6, 7). Additionally, Zn status determines whether the activation state of nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) (8). NFκB regulates the expression of pro-inflammatory cytokines (e.g., IL-1b, IL-6, IL-8, and TNF-α), chemokines and acute phase proteins (fibrinogen and CRP) (9); and hence the high prevalence of Zn-deficiency (10).

Copeptin was first identified by Holwerda in 1972 as a glycopeptide with 39 amino acids with leucine-rich core segment (11). It is derived from the arginine vasopressin (AVP) precursor with the equimolar ratio in the hypothalamus. While AVP is an unstable peptide with a short half-life

of 5 to 20 minutes; copeptin is a stable molecule that can be easily measured (12). Studies of copeptin levels in dialysis patients have shown that elevated copeptin concentrations is a potent biomarker in increasing the risk of mortality among HP (13, 14). Recent evidence shows that, elevated serum copeptin levels are associated with renal failure, increased renin angiotensin activity, water absorption, glomerular pressure, insulin resistance, inflammation and it is also negatively related to glomerular filtration rate (GFR) (12). Hence, a high serum copeptin may be used for the diagnosis and progression of CKD (15). Copeptin has recently been identified as a factor associated with inflammatory conditions in renal disease (12) and maybe involved in the anti-inflammatory role of Zn (6, 8, 16). We hypothesized that Zn status may be related to copeptin levels and to our knowledge, there is no evidence regarding the effects of Zn supplementation on serum copeptin in patients with diabetes and zinc deficiency. Moreover, previous studies on the effect of Zn supplementation on high sensitive CRP (hs-CRP) (17),and glucose homeostasis parameters (18) in HP are limited. Therefore, this double-blind randomized clinical trial study was performed to investigate the effects of Zn sulfate ($ZnSO_4$) supplementation on serum levels of copeptin and high sensitive C-reactive protein (hs-CRP), glycemic control and renal function in zinc-deficient diabetic hemodialysis patients (DHPs).

Materials and Methods

Participants

Forty-six diabetic hemodialysis patients (DHPs) with Zn-deficiency (age range: 35-62 years old) were recruited into the study between August-September 2019. Participants were screened and recruited at the Imam Ali Hospital Dialysis Center (Zahedan, Iran). Inclusion criteria included: a

serum Zn below the normal range [<80 μg/dL (19)], diabetic patients on hemodialysis treatment for at least 6 months, hemodialysis treatment 2 to 3 times per week.

Patients with inflammatory disease affecting serum inflammatory markers; or who had been hospitalized in the preceding 3 months; or who consumed any supplements containing antioxidants, any type of Zn, vitamins E and C, steroid and non-steroidal anti-inflammatory drugs one month before the study; or who were taking antibiotics for the past month and with infectious or feverish disease, were excluded.

Informed consent:

The Ethics Committee on Human Experimentation of Zahedan University of Medical Sciences, Iran approved the protocol of study (approval date: 21.07.2019; No. IR.ZAMUS.REC.1398.207). The subjects were informed of the study goals and procedures using a leaflet and a signed written informed consent was obtained. The Iranian Ministry of Health and Medical Education licensed supplements and all procedures complied with the relevant guidelines and regulations. This trial was also registered in the Iranian website (http://www.irct.ir) for registration of clinical trials (ID number: IRCT20190806044461N1).

Study Design:

To calculate the sample size, the standard formula suggested for parallel clinical trials by considering type one error (α) of 0.05 and type two error (β) of 0.20 (power = 80 %) were executed. Based on a previous study (17), the sample size was 20, but in order to reduce the problems related to the possible fall, more subjects were selected for the two groups. Participants were randomly divided into two groups; the Zn supplement group (n = 21) received a 220 mg/day Zn sulfate capsules (containing 50 mg Zn) based on previous studies (17, 20) and due to the high prevalence

of Zn-deficiency (10), and the control group (n=25) received a placebo capsule (220 mg/day corn starch), for 8 weeks. ***(Fig1***)

Alhavi Pharmaceutical Company (Tehran, Iran) produced and licensed the $ZnSO_4$ and corn starch capsules. Two experts contacted the patients once a week to ensure that they complied with all study protocols, correctly. In addition, people received a text message on their cell phones to take supplements daily to ensure adherence.

Randomization and Blinding: Participant allocation and block size were obtained using a table of random numbers. A nephrologist at the Dialysis Center of Imam Ali Hospital carried out the randomized allocation sequence, enrolled subjects, and assigned participants to intervention and placebo groups. The treatment allocation was double-blinded to all patients, clinical examiners, and other health care staff. Placebo capsules were identical in shape, color and size with supplements.

Anthropometric Measures:

At the beginning of the study and after 8 weeks, all participants underwent standard anthropometric measurements: dry weight after dialysis (Seca, Hamburg, Germany) and height. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared.

Assessment of Outcomes:

The ward nurse collected fasting, predialysis blood samples (10 mL) on the first day of the study and after the 8 weeks of intervention. Serum samples were stored at -70^{o} C until the end of the study.

Serum Zn levels were determined using atomic absorption spectrophotometry (AAS) (Shimadzu AA-670, Tokyo, Japan with analytical range: 1–150 µg/dL, http://www.ssi.shimadzu.com). The serum levels of copeptin (Bioassay Technology lab, No E1129Hu / 96 Test, China) and insulin (Monobind Inc, No 5825-300/ 96 test. USA) were measured using enzyme-linked immunosorbent

assay (ELISA) kits. Serum CRP concentrations were determined by immunonephelometry (MININEPH, Binding Site, Ltd., Birmingham, UK). Commercial kits were used to measure the levels of Blood Urea Nitrogen (BUN) and creatinine (*Cr*) (Pars Azmoun.co kit, Tehran, Iran). The measurements were performed by autoanalyzer (Hitachi 911, Japan), using enzymatic and colorimetric method. Additionally, an auto-analyzer machine (BT-1500, Italy) measured serum FBG by photometric enzymatic method using the standard kits (Pars Azmoon Inc., Tehran, Iran). Insulin resistance was estimated using the homoeostatic model assessment (HOMA-IR) method, using the following formula: [fasting insulin (µU/ml) - fasting glucose (mmol/l) / 22.5 (21). Quantitative insulin sensitivity check index (QUICKI) was calculated via following formula: **[**1/ (log fasting insulin [µIU/ml]) + log fastin glucose [mg/dl])] (21).

Statistical analysis:

The Statistical Package for Social Sciences (SPSS) software version 21 (SPSS, Inc. Chicago, IL, USA) was used for all statistical analyses. Kolmogorov–Smirnov test was used to determine normality of data distribution. Student's t-tests (for normal variables) and Mann-Whitney test (for non-parametric variables) were carried out for comparisons of baseline characteristics.

Paired t-test and Wilcoxon singed rank test were used for within group comparisons (after 8 weeks compared to baseline). Given that the baseline values for each variable could vitiate the relationships under study and affect the overall conclusion. Therefore, the effect of the intervention was examined by ANCOVA test adjusting for baseline values and other potential confounders. $P < 0.05$ was considered as the significant level.

Results

General characteristics:

Among 46 patients who were included, all of them completed the study (***Fig. 1***). As shown in **Table 1**, the mean age of participants in the control and Zn-supplemented groups were 54.1 years and 55.6 years, respectively. It is worth mentioning, the mean age of participants, dialysis adequacy (Kt/V) and duration of dialysis (months) were not significantly different between the ZnSO4 and placebo groups at baseline.

Effect of ZnSO4 on serum Zn Concentration

The initial mean concentration of serum Zn for participants in the control group was 68.2 ± 6.2 mg/dL SD, and for the Zn-supplemented group, it was 55.9 ± 8.02 mg/dL. Serum Zn concentrations in Zn-supplemented group illustrated significant increases as of day 56, from 55.9 ± 8.02 to 90.6 ± 15.71 mg/dL, which was statistically significant (***P < 0.001***). In the control group, Zn concentrations no significant changed in the control groups of day 56, from 68.2 ± 6.2 mg/dL to 68.5 ± 6.5 mg/dL (***P = 0.9***) (***Table 2***).

Effect of ZnSO4 on serum copeptin and hs-CRP levels:

As seen in **Table 2**, the circulating levels of copeptin decreased significantly throughout the study, (-16.5 ± 23.1 pg/mL change from baseline, P = 0.001) in the Zn group. Moreover, the difference was statistically significant compared with the control group following the supplementation (***P = 0.02***). Mean serum hs-CRP concentrations decreased after 8 weeks, from 8.73 ± 4.2 mg/L to 4.92 ± 2.07 mg/L, in the intervention group, which was significant (***P < 0.001***). However, in this same period, no significant change of hs-CRP was observed in the control group after 8 weeks (***P = 0.71***). The difference between serum CRP concentrations in the two groups was statistically significant at the end of trial (***P < 0.001***).

Effect of ZnSO4 on renal function:

There were no significant difference in serum BUN and Cr values between two groups at baseline (***P = 0.14, 0.19***; respectively). However, Serum BUN (***P* < 0.001**) and Cr (***P* < 0.001**) were significantly altered following ZnSO4 supplementation as compared to controls.

Effect of ZnSO4 on glycemic indices and anthropometric parameters:

As summarized in **Table 2**, FBG (***P* < *0.001***), BMI (**P < 0.001**) and body weight (***P* < 0.00**1) were significantly changed following 8 weeks in the group receiving ZnSO4 supplementation in comparison with the controls. While, receiving ZnSO4 supplement did not lead to statistically significant changes in serum insulin (***P = 0.55***), HOMA-IR (***P = 0.6***) and QUICKI (***P = 0.57***) in compared to controls.

Discussion

In this randomized double-blind controlled trial (RCT), we found the favorable effect of $ZnSO_4$ supplementation on serum copeptin, BUN and Cr levels in Zn-deficient DHPs. To the best of our knowledge, the present study is the first RCT examined the effects of $ZnSO_4$ supplementation on serum levels of copeptin. Given that copeptin is related to several metabolic factors, such as glomerular pressure, insulin resistance, inflammation and also Zn has been reported to alter the chronic inflammatory response (12), we hypothesized that copeptin serum concentrations may be related to Zn status.

Our findings indicate that copeptin was markedly reduced in the $ZnSO_4$ group. Whereas no significant difference was observed in the control group after 8 weeks. The relationship between copeptin levels and renal failure is well known (22) and is followed to Zn deficiency in dialysis patients (23). Koch et al., conducted a large observational study and they demonstrated that

copeptin serum concentrations were associated to the inflammatory markers, metabolic disorders and renal failure in critically ill patients (24). In line with this, other studies have shown that increased concentrations of copeptin are related to renal insufficiency, and copeptin is inversely related to estimated glomerular filtration rate (eGFR) (25, 26). The correlation between copeptin and urinary albumin/protein excretion is also worth to mention (26). Population-based studies have shown that copeptin is closely correlated with microalbuminuria (27). However, the exact mechanism relating to copeptin and GFR are not clearly understood (28). Therefore, our study focused on DHPs with Zn-deficiency and we investigated different variables in addition to copeptin, which are linked together. Our results showed that taking 220 mg $ZnSO_4$ (50 mg/day Zn) for 8 weeks in Zn-deficient DHPs, there were beneficial effects on renal function (reduced serum BUN and Cr). Additionally, Okamoto et al., demonstrated that pre-dialysis serum Cr level was independently related to responsiveness to Zn supplementation after 3 months in patients on maintenance hemodialysis (29). In summation, we speculate that Zn deficiency could be one of the factors affecting the copeptin and renal function in Zn-deficient DHPs.

In addition to the issues raised, we found that receiving 220 mg $ZnSO_4$ after 8 weeks lead to a significant reduction in hs-CRP in the intervention group in compared with the controls. However, Rashidi et al., reported that the administration of $ZnSO_4$ (220 mg for 8 weeks) caused a reduction of CRP in HP, but this occurrence was not statistically significant (17). This result is consistent with the findings of Scheurig et al., (30) and Peter et al., (31). However, Craig et al., (32) observed an inverse association between the concentrations of serum Zn and CRP which was in line with our results. One of the primary inflammatory mediators that regulates the expression of pro-inflammatory cytokines (e.g., IL1b, IL-6, and TNF-α), chemokines, and acute phase proteins (CRP and fibrinogen) is the NFκB (9, 33). The Zn status is involved in the activation state of NFκB (8).

The effects of Zn status on inflammation might have important impacts on endocrine outcomes and inflammatory biomarkers such as CRP. Interestingly, copeptin appears closely related to high levels of CRP (34-36). As a result, according to the above, it may be possible to find a relation between Zn and copeptin, which will require future research in this field.

Regarding the effects on glycemic indices, Gupta et al found that in patients with diabetic neuropathy who were taken $ZnSO_4$ (660 mg for 6 weeks), the change in pre and post therapy values of FBG were significant ($P < 0.0001$) and they concluded that $ZnSO_4$ supplementation helps in achieving better glycemic control, which is in line with our results. Several mechanisms, including interactions between Zn and the insulin receptor, insulin structural integrity and insulin signaling pathways, mediates Zn's effects on glycemic indices (37-39). Moreover, a recent evidence has shown that Zn plays an important role in insulin sensitivity by inducing the PI3K /Akt cascade that mediates insulin signaling and subsequent glucose access (38). It is also an important mediator of insulin accumulation and secretion from pancreatic beta-cells (40). However, the findings of the current study do not support the previous research and our finding is in agreement with a recent meta-analysis study by Capdor et al., it was stressed that Zn supplementation could significantly reduce fasting glucose while insulin changes were not significantly changed. In fact, they found that no substantial effects of Zn supplementation on insulin concentrations, despite the justification for the cellular impact of Zn on insulin signaling (41). In the study by Asghari et al., no change was observed in glycemic control, despite a substantial increase in the serum Zn level after 12 weeks of supplementation with 30 mg/d Zn gluconate in patients with DM (42). Moreover, Gómez-García et al., demonstrated that $ZnSO_4$ consumption (100 mg per day for 30 days) did not effect on insulin sensitivity (43) and also Kim et al., founded that 30 mg of Zn gluconate per day for 8 weeks in obese women did not change the insulin sensitivity index or the HOMA-IR values

(44), which is similar to our results. However, Payahoo et al., (45) reported that consumption of 30 mg per day of Zn gluconate in obese adult for 4 weeks caused a drop in HOMA-IR values, demonstrating the advantageous impact of the Zn supplementation on betterment of insulin resistance.

In the current study, we used $ZnSO_4$ for Zn supplementation. The most common forms of Zn compounds are Zn gluconate, $ZnSO_4$, Zn oxide, and Zn acetate. In previous studies, $ZnSO_4$ and Zn gluconate were cited most frequently (46). It is noteworthy that, among the participants in this study, no one reported side effects and considering that $ZnSO_4$ preserved epithelial and tissue integrity by boosting cell development, suppressing apoptosis and acting as an antioxidant that protects against free radical damage during inflammatory process (47). Perhaps, it might be concluded that $ZnSO_4$ is a practical and relatively safe supplement to achieve better results in the treatment of DHPs with Zn deficiency. However, like any other study, the current RCT has some limitations that should be noted. One of the limitations of this study is that despite the random assignment of participants to the two intervention and control groups, baseline values in some variables were significantly different between the two groups. Therefore, adjusted ANCOVA analysis was used to reduce this problem in this study. Additionally, small number of included patients and follow-up duration limited the current study. We suggest further studies to stress on the potential mechanisms of the relation of serum copeptin levels and Zn. In addition, other doses in different regions might affect the results.

Conclusion

Taken together, the results of this study illustrated that taking 220 mg $ZnSO_4$ (50 mg/day Zn) for 8 weeks in Zn-deficient DHPs, there were beneficial effects on copeptin, renal function and inflammation. Moreover, Zn administration might cause an enhancement in serum Zn

concentrations, leading to a reduction of FBG in DHPs with Zn-deficiency. However, the current findings did not support alteration in serum insulin.

Acknowledgments

This article is extracted from a master's degree thesis in nutrition. The authors are grateful to Research Vice Chancellor of Zahedan University of Medical Sciences for financial support, and to all patients for their sincere cooperation in this study.

Conflict of Interest

The authors declare that they have no competing interests.

Ethical Approval: The protocol of the study was approved by the Ethics Committee of the Zahedan University of Medical Sciences (approval date: July 2019; number: 9510)

Registration number (name of the registry and its URL). This trial was registered at the Iranian Registry of Clinical Trials (ID number: IR.ZAUMS.REC.1398.207).

Funding The study was supported by a grant from Zahedan University of Medical Sciences (Grant no.: 9510).

Authors' Contributions:

RH and FM contributed to the study design and concept. ESH and RH contributed to data collection. AMM and ARD contributed to data analyses. FM and MK contributed to the interpretation of data for the work. RH, FM, GAF and MJ contributed to drafting and reviewing the final manuscript. All read and approved the final manuscript for publication.

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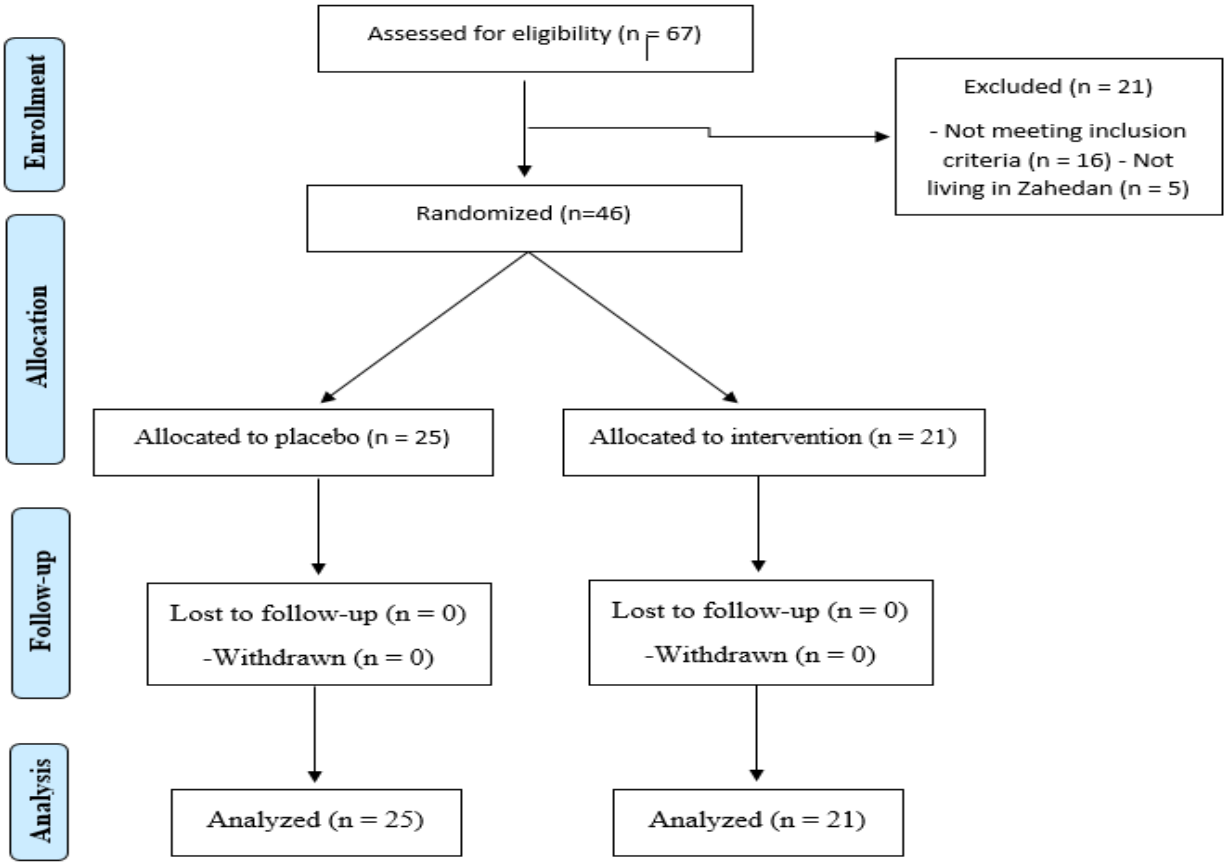

Fig 1. Enrollment and outcomes of the study

Table 1. Baseline characteristics of the study population, Mean ± SD or N (%)

		Groups		P
		Control (25)	ZnSO4 (21)	
Age, (yrs)		**54.1 ± 5.4**	**55.6 ± 7.3**	**0.11[M]**
Male		**14 (56)**	**8 (38.0)**	
Female		**11 (44)**	**13 (61.9)**	**0.08[*]**
Dialysis duration (months)		**41.4 ± 21.7**	**39.7 ± 24.7**	**0.44[M]**
Weight (kg)		**76.8 ± 15.4**	**66.2 ± 10.5**	**0.004[M]**
Education	**Illiterate**	**14 (56)**	**12 (57.1)**	
	Diploma	**8 (32)**	**5 (23.8)**	**0.38[*]**
	Bachelor	**3 (12)**	**2 (9.5)**	
	Master	**0 (0)**	**2 (9.5)**	
Kt/V		**1.0 ± 0.2**	**1.0 ± 0.3**	**0.75[T]**

Kt/V: dialysis adequacy: K – dialyzer clearance of urea. t –dialysis time. V –volume of distribution of urea; SD: Standard deviation

[*] Based on Chi-Square test

[T] Based on t-test.

[M] Based on Mann-Whitney test.

Table 2. Changes in copeptin, hs-CRP, glycemic indices and renal function throughout 8 weeks of intervention

		Control	ZnSO4	P
		Mean ± SD	Mean ± SD	
BMI (Kg/m^2)	**Before**	**27.9 ± 5.3**	**25.3 ± 3.4**	**0.03^{T}**
	After	**27.7 ± 5.1**	**26.0 ± 3.3**	**< 0.001§**
	Change	**-0.2 ± 0.7**	**0.7 ± 0.3**	**< 0.001^{T}**
	p$^{¥}$	**0.2**	**P < 0.001**	
Weight (Kg)	**Before**	**76.8 ± 15.4**	**66.0 ± 11.1**	**0.004^{M}**
	After	**76.2 ± 14.4**	**68.0 ± 10.8**	**0.001§**
	Change	**-0.5 ± 2.3**	**1.9 ± 0.9**	**< 0.001^{M}**
	p$^{£}$	**0.22**	**< 0.001**	
Zinc (µg/dL)	**Before**	**68.26 ± 6.2**	**55.9 ± 8.0**	**< 0.001^{T}**
	After	**68.5 ± 6.5**	**90.6 ± 15.7**	**< 0.001§**
	Change	**0.2 ± 9.8**	**34.7 ± 14.1**	**< 0.001^{T}**
	p$^{¥}$	**0.9**	**P < 0.001**	
BUN (mg/dL)	**Before**	**46.3 ± 9.5**	**44.1 ± 13.6**	**0.14^{T}**
	After	**46.9 ± 10.3**	**41.8 ± 13.8**	**0.001§**
	Change	**0.6 ± 2.6**	**-2.3 ± 3.2**	**< 0.001^{T}**
	p$^{¥}$	**0.26**	**0.002**	
Cr (mg/dL)	**Before**	**7.5 ± 2.1**	**6.7 ± 1.9**	**0.19^{T}**
	After	**7.8 ± 2.4**	**6.1 ± 2.2**	**0.01§**
	Change	**0.3 ± 0.9**	**-0.5 ± 1.5**	**< 0.001^{T}**
	p$^{¥}$	**0.06**	**0.07**	
Copeptin (pg/mL)	**Before**	**69.6 ± 54.6**	**77.0 ± 36.3**	**0.18^{M}**
	After	**69.3 ± 55.2**	**60.4 ± 34.8**	**0.33§**
	Change	**-0.3 ± 63.5**	**-16.5 ± 23.1**	**0.02^{M}**
	p$^{£}$	**0.69**	**0.001**	

Hs-CRP (mg/L)	**Before**	**7.3 ± 4.5**	**8.7 ± 4.2**	**0.44[T]**
	After	**7.8 ± 4.9**	**4.92 ± 2.07**	**< 0.001[§]**
	Change	**0.52 ± 0.4**	**-3.81 ± 2.71**	**< 0.001[T]**
	p[¥]	**0.71**	**P < 0.001**	
FBG (mg/dL)	**Before**	**181.6 ± 51.0**	**208.2 ± 85.4**	**0.23[M]**
	After	**184.1 ± 52.2**	**132.0 ± 47.9**	**< 0.001[§]**
	Change	**2.4 ± 7.6**	**-76.1 ± 54.9**	**< 0.001[M]**
	p[£]	**0.12**	**< 0.001**	
Insulin (µIU/ml)	**Before**	**26.2 ± 7.5**	**23.1 ± 4.3**	**0.01[M]**
	After	**26 ± 7.6**	**26.43 ± 8.9**	**0.65[§]**
	Change	**-0.1 ± 0.9**	**1.5 ± 18.0**	**0.55[M]**
	p[£]	**0.57**	**0.47**	
HOMA-IR	**Before**	**12.1 ± 6.1**	**11.6 ± 9.3**	**0.21[M]**
	After	**12.1 ± 6.0**	**8.86 ± 4.76**	**0.01[§]**
	Change	**0.0 ± 0.7**	**-3.2 ± 9.4**	**0.6[M]**
	p[£]	**0.77**	**0.21**	
QUICKI	**Before**	**0.27 ± 0.01**	**0.28 ± 0.02**	**0.11[T]**
	After	**0.27 ± 0.01**	**0.28 ± 0.01**	**0.04[§]**
	Change	**-0.0001 ± 0.002**	**0.002 ± 0.02**	**0.57[T]**
	p[¥]	**0.73**	**0.68**	

SD: Standard deviation; FBG: fasting plasma sugar; HOMA-IR: Homeostasis model assessment-insulin resistance; QUICKI: Quantitative Insulin Sensitivity Check Index; BUN: blood urea nitrogen; Cr: creatinine; Hs-CRP: High sensitive C-reactive protein.

[T] Based on t-test. [M] Based on Mann-Whitney test. [¥] Based on Paired Samples Test. [£] Based on Wilcoxon singed rank test. [§] Adjusted for baseline values based on ANCOVA.