Comparing the Effects of Eight-Week Zinc Supplementation and Yoga Exercise on Serum Apelin Level and Kidney Function Among Women with Type II Diabetes Mellitus

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Abstract

**Background:** Diabetes mellitus is a metabolic disorder associated with impaired serum lipid and glucose levels.

**Objectives:** The present study aimed to compare the effects of zinc supplementation, yoga exercise, and zinc supplementation plus yoga exercise on serum apelin level and kidney function among women with type II diabetes mellitus.

**Methods:** This was a four-group pretest-posttest quasi-experimental study. Thirty-two women were selected and randomly allocated to either of the following four groups: control; yoga exercise (thrice weekly for eight weeks); zinc supplementation; and zinc supplementation plus yoga exercise. Body composition, insulin resistance, and serum levels of glucose, insulin, creatinine, urea nitrogen, and apelin were measured before and after the study intervention. Data were analyzed through the one-way analysis of variance and the least significant difference post hoc test at a significance level of less than 0.05.

**Results:** There were significant differences among the study groups concerning the serum levels of glucose (P = 0.001), insulin (P = 0.012), apelin (P = 0.001), and urea nitrogen (P = 0.02) as well as insulin resistance (P = 0.016) after the intervention. However, groups did not significantly differ from each other concerning the serum level of creatinine (P = 0.264).

**Conclusions:** Eight-week yoga exercise and zinc supplementation are useful to women with type II diabetes mellitus.

**Keywords:** Type II Diabetes Mellitus, Yoga, Zinc Supplementation, Apelin, Kidney Function

1. Background

Diabetes causes impairment in insulin release and performance, thereby leading to an increase in glucose plasma levels (hyperglycemia) in addition to impaired carbohydrate, fat, and protein metabolism (1, 2). Fat tissue, as an endocrine organ, produces adipokines that control many physiologic functions (3). The identified hormones are derived from adipocytes or adipokines, including vaspin, adiponectin, resistin, and apelin, play an important role in type 2 diabetes (4, 5).

Apelin peptide is released from adipocytes, which is regulated with insulin. Recently, it has been demonstrated that it contributes to glucose homeostasis (5, 6). Yoga improves fat metabolism and the use of fat as the energy source for muscular glycogen and discharging it. Furthermore, yoga causes weight loss and plays a role in insulin resistance, endothelial factors, and releasing adipokines, and pro-inflammatory mediators (7, 8).

It has been established that blood glucose is decreased up to 16 hours after one session of aerobic exercise, where signaling pathways actively mediate transportation of glucose into the skeletal muscle (9, 10). Resistive exercises stimulate the kinase protein directing glucose-4 transferring protein (GLUT-4) to the cell surface and improve insulin sensitivity (11). Yoga is a combination of warm-up exercises, resistance movements, and aerobic exercises.

The expansions and contractions contained in Asana and Pranayama movements need glucose consumption (12). Kazemi et al. study suggested that 8 weeks of aerobic exercise in rats with type 2 diabetes increased plasma apelin, decreased blood fat, and improved type 2 diabetes (13). Chimkode et al. found that doing yoga exercises decreases fasting blood sugar and 2-hour blood sugar, and improves type 2 diabetes (14). Meanwhile, zinc reduces blood sugar by regulating metabolism and preventing nicotinamide adenine dinucleotide of phosphate oxidase (15, 16).

The catabolism of purines produces uric acid, which is...
excreted from the body. Keratin becomes creatinine and is urinated as waste through the kidneys. Increased serum level of these materials indicates the inability for urination from blood, which can result from a disorder or dysfunction in kidney performance (17). Kadoglu et al. observed that 12 weeks of aerobic exercise in patients with type 2 diabetes significantly reduced apelin levels (18). Aminilari et al. also found that aerobic exercise significantly decreases apelin and glucose (3).

2. Objectives

Few studies have been conducted on the effect of exercise on apelin and kidney performance in patients with type 2 diabetes. Thus the current study aimed to examine the effect of 8 weeks of zinc supplementation and yoga exercise on plasma apelin and kidney performance in women with type 2 diabetes.

3. Methods

The current study is a semi-experimental study with pre-test/post-test design. The study population consisted of 30- to 50-year old women with type 2 diabetes in Flavarjan. First, information on conducting the study was given by examining patients’ files, contacting them, sending forms, and installing posters in the clinic. The participants were selected based on the inclusion criteria, which were having type 2 diabetes; no history of cardiovascular, respiratory, cerebral, kidney, and hormone diseases; no regular exercise in the last 6 months. On the other hand, the exclusion criteria were lack of physical health, being absent more than two sessions, and lack of interest in continuing the study. Finally, 32 participants were selected and were divided into four groups, including yoga exercise (8), zinc supplement (8), combined (8), and the control groups (8) by simple random method.

The purposes, methods, benefits, and risks of the study were explained to the participants and written informed consent was also obtained. This study was approved by the Ethics Committee of Biomedical School of Islamic Azad University, Isfahan Branch (Khorasan) with the ethics code of IR.IAU.KHUISF.REC.1396.26 and received IRCT20170510033909N2 code from Iran’s Clinical Trials Registration Center. The subjects’ weight was measured by Snowa digital balance with 0.1 kg accuracy and their height was measured by Seca height-meter with 0.5 cm accuracy. Body mass index (BMI) was determined by dividing body weight (kg) by square height (m²). Finally, the waist and hip values were measured and then the waist size was divided by the hip value to determine its ratio.

3.1. Exercise Plan

The intervention group did yoga exercises three sessions a week and every session lasted 75 minutes. Yoga exercises were comprised of Asana movements, which were warm-up exercises for 45 minutes. Then Pranayama movements were done, which were practiced in a sitting form with rhythmic inhalation and exhalation. The final step was meditation for 15 - 20 minutes. This step was done after Pranayama movements and involved sleeping in solitude, rhythmic respiration, isometric contractions of bid muscles, bending and stretching, abandoning, and concentration (19).

3.2. Supplementation

This step consisted of the use of zinc supplements for 8 weeks in the form of one 50 mg zinc sulfate tablet after each meal. For the control group and aerobic exercise group, capsules containing corn starch powder were prepared. Blood samples were collected in two steps: 24 hours before beginning the first session and 48 hours after the last session, after breakfast and during relaxation, and each time 10 mL from the left hand’s anterior vein. Apelin plasma was measured by Human Apelin Kit made in China by ELISA method. Serum glucose, creatinine, and BUN were measured by glucose oxidase method, and Zinc was measured by Randox method using Pars Azmoun Kit. Insulin was measured by Cuba’s Kit made in England by immunoassay method. To measure insulin resistance, HOMA-IR formula was employed. This formula is HOMA-IR = fasting insulin (µU/mL) × fasting glucose (mmol/L) divided by 22.5.

The samples were placed in sterile tubes, and then blood serum was separated by centrifugation (3000 rpm for 10 minutes) and further freezing at 70°C until measurement. After collecting samples in the post-test step and according to pre-test, all blood samples were prepared and experiments were done according to the protocol. Furthermore, the subjects of the experimental group were asked not to do any exercise or long jogging until 24 hours after the exercise period. The subjects were also asked not to use capsules 24 hours before blood collecting. The SPSS 21 software was used for data analysis. Paired t test was employed to examine intragroup difference, one-way variance analysis (ANOVA) was used for intergroup comparison, and LSD test was used to determine the difference. The P value ≤ 0.05 was considered significant.

4. Results

This study was conducted on 32 women with type 2 diabetes. Table 1 presents the result of ANOVA test for general characteristics of subjects, including age and BMI.
The results of ANOVA test in Table 2 revealed that there is a significant difference in weight ($P = 0.001$) and BMI ($P = 0.001$) in the four groups; however, no significant difference was seen for the waist to hip ratio ($P = 0.1$).

Table 2 also presents the comparison of the weight, BMI and waist to hip ratio before and after exercise in the four groups.

The results of ANOVA in Table 3 indicated that there is a significant difference in insulin ($P = 0.01$), insulin resistance ($P = 0.01$), apelin ($P = 0.001$), and nitrogen ($P = 0.002$) in the four groups; however, no significant difference was seen in glucose ($P = 0.1$) and creatinine ($P = 0.2$).

Based on the results of post-hoc test in Table 4 in terms of insulin, there is a significant difference between the control and combined groups ($P = 0.002$), between the supplement and combined groups ($P = 0.008$), and between the yoga and combined groups ($P = 0.023$); however, there is no significant difference between the control and yoga groups ($0.35$) as well as between the control and supplement groups ($0.49$).

There is also a significant difference between the control and yoga groups ($P = 0.006$) and between the control and combined groups ($P = 0.005$) in terms of insulin resistance. Moreover, the results of follow-up test on apelin revealed a significant difference between the control and yoga groups ($P = 0.01$), between the control and supplement groups ($P = 0.001$), between the supplement and combined groups ($0.001$), as well as between yoga and combined groups ($0.001$). However, there was no significant difference between the control and combined groups ($P = 1.0$) and between supplement and yoga groups ($P = 0.69$).

Table 1. Results of ANOVA Test for Age and BMI in the Four Groups

| Group            | Yoga       | Zinc      | Combined  | Control    | F      | P Value |
|------------------|------------|-----------|-----------|------------|--------|---------|
| Age, y           | 47.37 ± 1.62 | 46.87 ± 3.31 | 44.42 ± 4.99 | 44.62 ± 3.24 | 1.39   | 0.26    |
| BMI, kg/m²       | 34.41 ± 4.93 | 32.36 ± 2.13 | 29.35 ± 5.37 | 32.25 ± 1.88 | 1.17   | 0.33    |

5. Discussion

The results of the current study indicated that 8 weeks of zinc supplementation and yoga exercise significantly decreased glucose in comparison to the control group; however, the combined group did not show any significant difference compared with the control group. Increased blood glucose through enhancing insulin sensitivity increases the number of GLUT-4 and elevates oxidative enzymes in skeletal muscles, therefore blood glucose is reduced. Furthermore, the contractions and expansions contained in Asana and Pranayama movements, which are composed of relaxation, deep breathing, bending, and stretching, stimulate pancreas cells directly; in turn, it increases insulin release and glucose metabolism. In addition, yoga enhances glucose absorption, raises blood flux in muscles, decreases insulin resistance, and amplifies insulin sensitivity contributing to lowering blood sugar levels (20).

Reduction of insulin resistance occurs by mechanisms such as increasing receptors of insulin signals such as Insulin Receptor Substrate-1, enhancing glucose transferring protein-4 (GLUT-4), amplifying the activity of glycogen synthase and hexokinase enzymes in the skeletal muscle, reducing the release and increasing the use of free fatty acids of plasma, increasing the use of glucose, and changing the muscle structure (21). This is consistent with the Rai et al.’s study on the effect of yoga in adults with type 2 diabetes indicating that yoga exercise decreases glucose (22). This has been due to the same exercises. In Chimkode et al.’s study on the effect of 6 months of yoga exercise on the blood sugar in patients with type 2 diabetic, it was found that yoga reduced blood sugar (14). This is because subjects were similar.

Rai et al. (22) tested the effect of 6 months of yoga exercise on fasting blood sugar in patients with type 2 diabetes. They concluded that yoga significantly reduced blood sugar levels. This is because yoga exercises were identical (Pranayama movements). In Navaei et al.’s study (23) on the effect of zinc supplement on glucose levels in men with type 2 diabetes, it was found that zinc supplement does not affect the blood glucose status. This is not consistent with the findings of the current study (24), which is due to the difference in gender and age of subjects as well as the time of using the supplement.

In the current research, yoga exercise and zinc supplement (combined group) significantly affected the insulin level. Considering insulin resistance, the control group showed a significant difference compared with all three
Table 2. Results of Paired t Test and ANOVA Test Before and After Exercise in the Four Groups

| Variable/Step                | Group          | P Value (ANOVA) |
|-----------------------------|----------------|-----------------|
|                            | Yoga           | Zinc            | Combined        | Control         |
| Weight, kg                  |                |                 |                 |                 |
| Before                      | 80.87 ± 9.15   | 82.68 ± 5.89    | 71.81 ± 13.22   | 80.02 ± 1.47    | 0.07            |
| After                       | 78.75 ± 8.79   | 80.71 ± 6.10    | 69.96 ± 13.41   | 79.91 ± 1.74    | 0.07            |
| P (paired t test)           | 0.006          | 0.01            | 0.000           | 0.49            | -               |
| **BMI, kg/m**²              |                |                 |                 |                 |
| Before                      | 32.43 ± 4.93   | 32.36 ± 2.13    | 29.35 ± 5.37    | 32.25 ± 1.88    | 0.33            |
| After                       | 31.57 ± 4.72   | 31.58 ± 2.20    | 28.60 ± 5.51    | 32.20 ± 1.87    | 0.27            |
| P (paired t test)           | 0.006          | 0.01            | 0.000           | 0.40            | -               |
| Waist to hip ratio          |                |                 |                 |                 |
| Before                      | 0.77 ± 0.04    | 0.88 ± 0.08     | 0.83 ± 0.05     | 0.86 ± 0.05     | 0.006           |
| After                       | 0.68 ± 0.25    | 0.86 ± 0.05     | 0.80 ± 0.00     | 0.93 ± 0.05     | 0.005           |
| P (intragroup paired t test)| 0.3            | 0.1             | 0.08            | 0.003           | -               |

groups. Exercise enhances insulin sensitivity; meanwhile, yoga exercises can be classified in resistive exercises. Researchers have declared that although BMI may have a positive relationship with improved insulin performance, resistive exercises seem to improve insulin sensitivity and HbA1c even without increasing BMI mainly by enhancing GLUT4 content and insulin signaling (19).

Therefore, the result obtained for insulin resistance can be partly justified. Insulin is a molecule produced by pancreas islands beta cells. Adding zinc to insulin structure enhances the insulin’s ability to connect to its receptors. Furthermore, zinc functions in the synthesis of insulin receptors, thereby making muscles and fat cells use glucose. Impairment in the ability of island cells in patients with type 2 diabetes in producing insulin and using glucose has been reported in zinc deficiency in these patients (20). Insulin resistance refers to the diminished normal performance of muscular cells for absorbing glucose in response to the insulin released from pancreatic beta cells. Insulin resistance is considered the cornerstone of metabolic syndrome. Meanwhile, studies suggest that yoga exercises improve glucose homeostasis and increase insulin sensitivity (7).

Zinc has an important role in the performance of pancreatic beta cells, insulin performance, glucose homeostasis, diabetes pathogenesis, and its complications. Zinc supplement enhances the activity and level of key antioxidant enzymes and proteins, while significantly reduces fat peroxidation. Zinc has also an important role in glucose and fat metabolism (25). In Zarei et al. study on the effect of three aerobic-resistive programs with different intensities for 12 weeks on metabolic control and visfatin levels in men with type 2 diabetes, it was indicated that aerobic exercises do not make any significant difference in insulin levels and insulin resistance of patients (25), which is consistent with the findings of the current research, because of the same exercises.

The findings of the current study are also consistent with the results that showed no changes in insulin resistance in spite of diminished glucose level. In Samadiyan et al.’s study on the effect of 12 weeks of combined exercises (aerobic-resistive) on resistin serum levels and glycemic indices in obese women with type 2 diabetes, it was found that insulin resistance was decreased and insulin did not alter (26), which is not consistent with the current study in terms of insulin resistance. This may be because of weight loss and a longer exercise period. In the current research, plasma apelin level was decreased in supplement and yoga groups. Insulin is the major regulator of apelin, where apelin increases glucose absorption. Research suggests that apelin is effective in glucose homeostasis, increasing insulin, and decreasing blood sugar. Accordingly, it has a relationship with insulin resistance, obesity, and type 2 diabetes. It also affects energy metabolism and insulin sensitivity. Indeed, insulin directly affects apelin production and release as a strong apelin regulator (27).

Indeed, it seems that apelin level is decreased in patients with diabetes due to a reverse mechanism, which may occur with exercise. Another possible mechanism for justifying apelin reduction after aerobic exercise can be attributed to BMI. A positive and significant relationship has been reported between apelin and physical activity. Also, the prominent role of apelin in controlling the inflammation by preventing Nuclear Factor Kappa-B has been
### Table 3. Results of Paired t Test and ANOVA Test for Glucose, Insulin, Insulin Resistance, Apelin, Creatinine, and Blood Urea Nitrogen Before and After Exercise in the Four Groups

| Variable/Step   | Group                  | P Value (ANOVA) |
|-----------------|------------------------|-----------------|
|                 | Yoga                   | Zinc            | Combined       | Control       |
| Glucose, mg/dL  | Before                 | 181.37 ± 37.14  | 194.25 ± 6.29  | 146.37 ± 17.57 | 161.25 ± 4.92 | 0.000 |
|                 | After                  | 156.25 ± 21.27  | 175.50 ± 5.39  | 139.75 ± 14.76 | 160.25 ± 9.25 | 0.000 |
|                 | P (paired t test)      | 0.001           | 0.000          | 0.001          | 0.71           | -    |
| Insulin, mU/L   | Before                 | 12.45 ± 1.39    | 11.50 ± 2.76   | 13.65 ± 2.70   | 9.76 ± 0.39    | 0.005 |
|                 | After                  | 11.50 ± 1.28    | 10.99 ± 2.62   | 10.35 ± 3.07   | 9.65 ± 0.37    | 0.35  |
|                 | P (paired t test)      | 0.02            | 0.001          | 0.41           | 0.6            | -    |
| Insulin resistance | Before             | 5.62 ± 1.48    | 5.48 ± 1.17    | 4.83 ± 0.45    | 3.85 ± 0.26    | 0.05  |
|                 | After                 | 4.37 ± 0.22    | 4.74 ± 1.01    | 3.55 ± 0.99    | 3.80 ± 0.36    | 0.014 |
|                 | P (paired t test)      | 0.02            | 0.001          | 0.007          | 0.06           | -    |
| Apelin, ng/mL   | Before                 | 2.87 ± 0.32    | 2.51 ± 0.47    | 2.30 ± 0.42    | 2.50 ± 0.07    | 0.018 |
|                 | After                 | 2.38 ± 0.28    | 2.11 ± 0.14    | 1.98 ± 0.37    | 2.17 ± 0.06    | 0.025 |
|                 | P (paired t test)      | 0.001           | 0.001          | 0.001          | 0.001          | -    |
| Creatinine, mg/dL | Before              | 0.97 ± 0.04    | 0.88 ± 0.08    | 0.88 ± 0.08    | 0.97 ± 0.04    | 0.011 |
|                 | After                 | 0.96 ± 0.05    | 0.92 ± 0.04    | 0.92 ± 0.04    | 0.96 ± 0.05    | 0.22  |
|                 | P (paired t test)      | 0.6             | 0.08           | 0.08           | 0.68           | -    |
| Blood Urea Nitrogen, mg/dL | Before       | 13.75 ± 4.83  | 9.50 ± 2.92    | 9.50 ± 2.92    | 13.75 ± 4.83   | 0.046 |
|                 | After                 | 12.37 ± 4.47   | 8.87 ± 2.58    | 8.07 ± 3.10    | 12.75 ± 3.95   | 0.15  |
|                 | P (paired t test)      | 0.001           | 0.01           | 0.17           | 0.018          | -    |

In the current research, changes in insulin resistance were consistent with variations of apelin. Both variables showed a significant decrease in yoga and supplement groups, justifying the obtained results.

In Kazemi et al. study on the effect of 6 weeks of aerobic exercise on apelin plasma concentration and insulin resistance in rats with type 2 diabetes, it was determined that aerobic exercise elevates the apelin plasma concentration in patients with this type of diabetes. This has not been in line with the findings of the current research (29). This dif-
ference can be ascribed to the difference in the type of subjects and shorter exercise period. In Mohebi et al. study (2013) on the effect of 8 weeks of medium intensity aerobic exercise on apelin plasma levels in women with type 2 diabetes, it was found that aerobic exercise significantly reduced the apelin plasma concentration, which is consistent with the current study. In NIKsersesht et al. study (2015) on the effect of 12 weeks of non-linear resistive and periodic aerobic exercise on apelin serum levels in obese men, no significant difference was found in apelin concentration (4), which is not consistent with the current research. This has been because of different subjects and type of exercise.

In the current research, kidney performance was examined in terms of blood urea nitrogen (BUN) and creatinine. The BUN is the final product of catabolism of proteins in the body and creatinine is a urinated chemical, which is resulted from keratin metabolism. They are among the main indices of kidney diseases. Increased serum levels of these materials show a diminished clearance and inability of kidneys to urinate these materials from the blood. Therefore, the serum level of these materials can be used as an index for assessing kidney performance (17). The BUN was decreased first in the yoga group and then in the zinc supplementation group. Although it was increased in the combined group, there was no significant change in the creatinine level.

In Samavatisharif and Siyavashi study (17) on the effect of 10 weeks of aerobic-resistive exercise on glomerular filtration and serum indices of kidney performance in men with type 2 diabetes, it was found that aerobic-resistive exercise improves kidney filtration and serum level of creatinine and BUN, which is consistent with the current research. However, it is not in agreement with the current research in terms of creatinine. This is because the subjects and type of exercise were different. In El-Ashmony et al. study (29) on the effect of 8 weeks of using zinc supplementation on blood sugar, blood lipids, and kidney performance; it was revealed that Zinc supplements reduced blood sugar and improved kidney performance in patients with type 2 diabetes, which is consistent with the current research. This similarity might be attributed to the fact that the weeks of use were identical.

5.1. Conclusions

Eight weeks of yoga exercise and zinc supplementation can be useful for patients with type 2 diabetes by decreasing apelin and glucose levels. It is suggested that such programs should be designed and implemented with a larger sample size and three sessions a week.

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Footnotes

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