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Effect of L-arginine and Selenium on Metabolic Features, Insulin Resistance and Hepatic Function Tests in Obese Women

Bahram Pourghassem Gargari[1], Mohammad Alizadeh[2*], Abdolrasoul Safaeiyan[3] and Rassoul Zarrin[2]

[1]Nutrition Research Center, Department of Biochemistry and dietetics, School of Nutrition, Tabriz University of Medical Sciences, Tabriz, Iran. [2]Food and Beverages Safety Research Center, Department of Nutrition, School of Medicine, Urmia University of Medical Sciences, Urmia, Iran. [3]Department of Vital Statistics and Epidemiology, Tabriz University of Medical Sciences, Tabriz, Iran.

Abstract: To investigate the beneficial effect of L-arginine and selenium along with hypocaloric diet on metabolic features, insulin resistance and hepatic function tests this study were conducted on women with central obesity. The randomized, double-blind, placebo-controlled trial was undertaken among 68 premenopausal women with central obesity. After 2 weeks of run-in period of an isocaloric diet, participants were randomly assigned into four groups of hypocaloric control diet (HCD), L-arginine (5 g / d) + HCD, selenium (200 µg / d) + HCD, or L-arginine + selenium + HCD for 6 weeks. The following variables were assessed at baseline (start of intervention) and 3 and 6 weeks after intervention: Waist circumference (WC), systolic blood pressure (SBP), diastolic blood pressure (DBP), fasting serum concentrations of triglyceride (TG), High-density lipoprotein-cholestrol (HDL-C), fasting blood sugar (FBS), insulin, homeostasis model of insulin resistance (HOMA-IR), alanine aminotransferase (ALT) and aspartat aminotransferase (AST). When t-test was used for data analysis, HCD reduced SBP (P = 0.009) and TG (P = 0.05); HCD + L–arginine reduced WC more than HCD (P = 0.008); HCD + Selenium reduced fasting serum concentration of ALT (P = 0.007); and selenium reduced fasting serum concentrations of insulin (P = 0.05) and HOMA – IR (P = 0.04), after 6 weeks of interventions. The study showed beneficial effects of L-arginine on central obesity, selenium on insulin resistance and hepatic function and HCD on blood pressure and serum lipid profile.

Keywords: Central obesity, hypocaloric diet, insulin resistance, L-arginine, metabolic syndrome, selenium.

INTRODUCTION

Central obesity is strongly associated with insulin resistance and non-alcoholic fatty liver diseases (NAFLD) [1]. Also central obesity is the main predictive determinant of metabolic syndrome, which includes hypertension, hyper triglyceridemia, elevated fasting glucose, and low serum concentration of HDL-C [1]. So far, there have been no strong strategies to reduce the central obesity and metabolic syndrome. It is therefore that, identifying new ways which can increase the effect of hypocaloric diets will be extremely beneficial for human health.

The oral supplementation of high dose of L-arginine, a precursor of nitric oxide (NO), has shown beneficial effects on some components of metabolic syndrome in animal models and type 2 diabetic humans [2-4]. While there are no studies undertaken on the effects of low dose of L-arginine in obese women. Physiological levels of NO (25 – 35 µmol/l), produced by endothelial (eNOS) and neuronal nitric oxide synthase (nNOS), enhanced glucose and fatty acid oxidation and inhibited glucose and TG synthesis [5]. Since NO is a free radical molecule, pathological levels of NO (13 – 15 folds of physiological level), produced by inducible nitric oxide synthase (iNOS), could disturb the cell action [5]. Obesity enhances expression of iNOS and production of NO. Although the blood concentration of NO among overweight people is near the maximum end of physiological range, due to increased production of free radicals, its bioavailability is low [6].

Selenium as an antioxidant may increase the bioavailability of NO, moreover selenium decreased pathological production of NO by iNOS, *in vitro*, and its deficiency enhanced iNOS expression [7, 8]. Despite insulin-like action of selenium [9], its supplementation in US population with its high serum concentration, increased risk of diabetes [10-12]. The effect of selenium supplementation on insulin resistance in communities with low serum concentration of selenium like Tabriz [13] is unknown.

The main interventions undertaken to control obesity and metabolic syndrome were related to changes made in lifestyle factors like dietary habits and weight control [14], so Hypocaloric control diet (HCD) was considered as a basic diet. This is the first time that the effect of low doses of L-arginine and / or selenium and HCD on metabolic features is studied in females with central obesity.

*Address correspondence to this author at the Food and Beverages Safety Research Center, Department of Nutrition, School of Medicine, Urmia University of Medical Sciences, Serow Highway, Nazloo, PO Box: 5756115111, Urmia, Iran; Tel: 00984412752372; Fax: 00984412780800; E-mail: alizade85@yahoo.com

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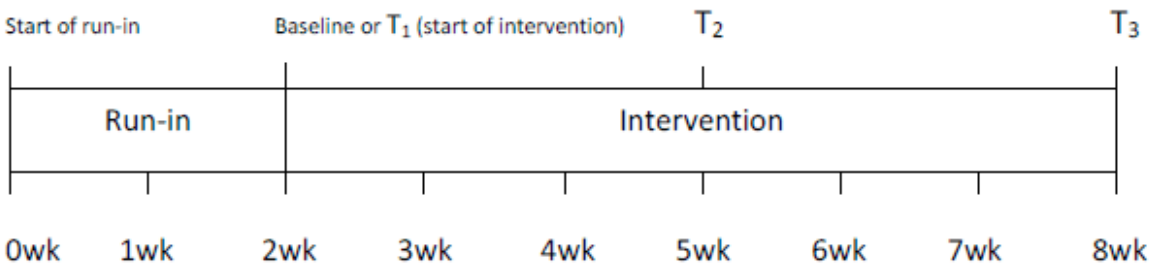

Fig. (1). The diagram of study design.

MATERIALS AND METHODS

The study was approved by the ethics committee of Tabriz University of Medical Sciences, and registered at www.irct.ir (irct ID: irct138712101720N1). A written informed consent was taken from all the subjects.

This research was a randomized, double-blind, placebo-controlled study with 8 week follow-up period. Inclusion criteria were: pre-menopausal women aged 20 – 50 years, waist circumference (WC) > 88 cm and weight maintenance (±2 kg) during the previous 6 months. Eighty four women were enrolled, 16 women did not complete the study and 68 women remained for the analysis (17 in each arm). Detailed information on the selection of participants has been given elsewhere [15].

The energetic needs of all participants were calculated individually by the formula from the Food and Nutrition Board of the Institute of Medicine [16]. The participants consumed an isocaloric diet for 2 weeks in the run-in period and HCD in the intervention period. HCD was 500-kcal less than caloric needs of participants. The macronutrient content of both diets was 55% carbohydrate, 30% fat, and 15% protein. Participants were being visited every week for 20–30 minutes to explain the advantages of diets and to train them how to perform their planned diet and to write "food diaries". Each participant had to write her 3-day physical-activity and diet records before entering the run-in period as well as before, in the middle, and at the end of the intervention period. Participant compliance was evaluated by weekly visits and the 3-day food diaries. Subjects who did not complete ≥ 80% of the planned diets for two consecutive weeks were excluded from the study (n = 12). Supplements of L-arginine and selenium were offered weekly. Participants consumed 96 ± 3.5% of the administered pills.

We planned a run-in period to obtain detailed information on the study population and to standardize macronutrient consumption. Among individuals eligible to enter the study, only those who maintained their weight at the end of the run-in period using an isocaloric diet based on the equation, were selected. After 2 weeks of the run-in period on an isocaloric diet, participants were randomly allocated to 4 intervention groups for 6 weeks: (1) HCD supplemented with a placebo of L-arginine and a placebo of selenium; (2) HCD supplemented with L-arginine (5 g / day) and a placebo of selenium; (3) HCD supplemented with selenium (200 µg / day) and placebo of L-arginine; and (4) HCD supplemented with L-arginine and selenium. We needed matched and random-allocated groups, so we used factor analysis and distributed all of the participants in four groups. Participants in each group were similar according to metabolic features and general characteristics. Participants in each group were then randomly allocated to four study groups. We repeated random allocation several times and selected the most homogenous groups. The dependent variables were measured at baseline (start of intervention), in the middle, and at the end of the intervention. Subjects were asked not to vary their common physical activities during the study. Fig. **1** shows the diagram of study design.

L-arginine (5 g / day) was administered as two 1-g L-arginine-hydrochloride tablets (Pooyan Nutrition Company, Tehran Iran, a joint venture with Mass Global Nutrition, Toronto, Canada; t.i.d.) with meals. Selenium (200 µg / day) was administered as a selenium-enriched yeast tablet (Nature Made; Pharmavite LLC, San Fernando, CA, USA) one tablet / day, 2 h after one of the meals (and after L-arginine). The main composition of the placebo tablets was starch and lactose.

All measurements were carried out by the unchanged investigator, and the unchanged tool in the first and follow-up evaluations. WC was measured (to the nearest 0.1 cm) at the narrowest point without pressure to the body surface by the light clothing using a tape measure. Blood pressure was measured thrice after sitting for 15 min.

Levels of fasting blood glucose (FBG), HDL-C and TG were measured enzymatically (ParsAzmoun, Tehran, Iran). Plasma levels of insulin were measured by a human insulin enzyme-linked immunosorbent assay (ELISA) test kit (Diaplus, San Francisco, CA, USA) according to manufacturer instructions. Insulin resistance was calculated on the basis of the homeostasis model assessment of insulin resistance (HOMA-IR) [17]. Both alanine (ALT) and aspartat aminotransferases (AST) were measured by International Federation of Clin.ical Chemistry (IFCC) method without adding prydoxal phosphate (Pars Azmoon kit, Tehran, Iran). Levels of nitrites/nitrates were measured concurrently using the Griess reaction [18].

Additional covariate information was obtained by questionnaires. A participant was characterized as overweight if the body mass index (BMI) was > 25 kg / m^2. Definition of the metabolic syndrome was based on the criteria set by the Adult Treatment Panel III [19].

Table 1. Effect of interventions on metabolic features by nested M-ANOVA repeated measurements of multi-factor model after controlling for WC.

	Interventions												P_{HCD}	P_{Se}	P_{Arg}	P_{Arg+Se}
	HCD			HCD +Arg			HCD +Se			HCD +Arg +Se						
	T_1 (mean ±SE)	T_2 (mean ±SE)	T_3 (mean ±SE)	T_1 (mean ±SE)	T_2 (mean ±SE)	T_3 (mean ±SE)	T_1 (mean ±SE)	T_2 (mean± SE)	T_3 (mean± SE)	T_1 (mean± SE)	T_2 (mean ±SE)	T_3 (mean± SE)				
WC (cm)	92.7± 1.7	89.8± 1.9	88.4± 1.8	92.2± 2.2	86.9± 2.3	84.8± 2.5	91.5± 1.8	87.9± 1.6	86.1± 1.5	91.9± 2.3	87.1± 2.5	85.9±2.7	0.000	0.93	0.60	0.79
SBP (mmHg)	121±3	115.4± 3	111.3± 3.5	120± 2.4	113.3± 2.2	116.7± 2.2	120.2± 3.8	115.1± 3.2	118±3.6	120±2.1	120.7± 2.6	120.6± 2.6	0.1	0.12	0.41	0.50
DBP (mmHg)	77.9± 2.1	78.7± 1.7	76.1± 2.1	77.1± 2.6	74.7± 2.3	76.7± 2.1	77.2± 2.3	74.6± 1.9	74.1± 3.1	78±2.1	78.3± 2.4	79.6±2.4	0.93	0.99	0.40	0.22
FBS (mg/dl)	91.8± 2	97±2.6	92.2± 2.3	90.7± 2.2	98.5± 2.5	93.5± 2.8	91.5± 1.9	89.6± 3.7	93.3± 2.7	91.8± 1.8	94.4± 2.1	93±2.4	0.02	0.12	0.43	0.86
TG (mg/dl)	160.6 ±13	154± 14.3	141± 13.6	160.3± 20.1	137.6± 20.6	150± 19.9	160.2± 16.8	158.9± 12.8	159.2± 14.2	160.5± 17.4	144.8± 18	154.2± 15.2	0.86	0.80	0.85	0.92
HDL-C (mg/dl)	44.6± 1.2	46.2± 1.3	45.3± 1.4	45.8± 1.1	46.5± 1.1	46.9± 1.3	45.5± 1.6	46.1± 1.3	45±1.4	44.6± 1.6	44.2± 1.5	43.2±1.9	0.70	0.40	0.95	0.37
Insulin (μIU/ml)	18.8± 1.1	23.9± 2.3	19±1.5	18.3± 2.9	16.3± 2.9	17.1± 3.4	18.5± 2.8	15±1.9	15.1± 1.4	18±2.2	15±1.5	16.7±1.4	0.98	0.05	0.43	0.25
HOMA-IR	4.3±.3	5.6±.5	4.4±.4	4.1±.6	3.9±.7	4±.8	4.3±.7	3.4±.5	3.5±.4	4.1±.5	3.5±.3	3.8±.3	0.83	0.04	0.57	0.38
AST (U/l)	21.2± 1.6	23.9± 2.2	18.2± 1.9	21±2.2	22±1.7	19.4± 2.2	20.9± 1.1	20.2± 1.4	19.1±1	20.3± 1.5	20.5±2	23±2.5	0.41	0.28	0.45	0.87
ALT (U/l)	21.5± 3.2	22.6± 3.1	16.2± 2.5	21.6± 6.6	21.3±3	17.5± 4.9	21.2± 3.1	20.6± 4.7	15.1± 2.4	21.5± 2.8	23±3.6	22.8±4.6	0.41	0.95	0.60	0.88
NO_x (μmol/l)	29.3± 8.2	37.2± 9.4	33±7.4	29±8	27.4± 6.5	29.6± 10.5	29.1± 16.3	21.6±1 4.3	21.1± 11.2	29.4± 14.2	29.3± 12.9	23.5± 13.5	0.74	0.47	0.89	0.85

Values are means ± SE. HCD: Hypocaloric Control Diet; Arg: L-arginine; Se: Selenium; T_1: Before intervention; T_2: Three weeks after intervention; T_3: Six weeks after intervention; WC: Waist Circumference; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; FBS: Fasting Blood Sugar; TG: Triglyceride; HDL: High Density Lipoprotein; HOMA – IR: Homeostasis model of insulin resistance; AST: Aspartate Amino Transferase; ALT: Alanine Amino Transferase; NO_x: Nitrite/Nitrate.

With a $1-\beta = 95\%$ and $1-\alpha = 95\%$, the Maximum sample size was estimated from WC and evaluated to be 17 persons for each group.

Two methods were applied for statistical analyses. In the first method, multifactor model of nested M-ANOVA repeated measurements were used by Minitab Package (v13). In this model the concurrency of analyses instead of multiple comparisons minimized the probability of false-positive results.

In the second method, a paired *t*-test or its nonparametric equivalent (Wilcoxon test) was used for comparing the amount of variables in different times within groups. Furthermore, an independent *t*-test or the Mann–Whitney U-test was used for comparing the percentage changes in variables during different times (T3–T1, T2–T1 and T3–T2) in groups with a change in the HCD group. Histograms were used to recognize normal distributions. These analyses were conducted using SPSS 13.0 (SPSS, Chicago, IL, USA).

χ^2 test and independent *t*-test were applied to find significant differences in baseline (start of intervention) values among intervention groups. For appropriate variables, subclasses of variables were merged and then the χ^2 test was used and two-tailed $P \leq 0.05$ was considered significant.

RESULTS

The effects of interventions on metabolic features by nested M-ANOVA repeated measurements model were outlined in Table **1**. There were no significant differences among basal (before intervention) measurements in 4 groups. (Not shown in Table **1**).

After 6 weeks that L-arginine and selenium administration was added to HCD, the following results were obtained (Table **1**): 1) Selenium significantly reduced fasting serum concentrations of insulin ($P = 0.05$) and HOMA-IR ($P = 0.04$); 2) HCD significantly reduced WC ($P < 0.001$) and systolic blood pressure (SBP) ($P = 0.05$), but the association of HCD and SBP did not remain significant after controlling for WC; 3) HCD significantly increased FBS after 3 wk and returned it to basal levels after 6 wk ($P = 0.02$); and 4) no significant effect of HCD, L-arginine, selenium and L-

Table 2. Effect of interventions on metabolic features with in groups by paired t-test or willcaxon analysis.

Variables	Interventions											
	HCD			HCD+Arg			HCD+Se			HCD+Arg+Se		
	$P_{T2,T1}$ (change percent)	$P_{T3,T2}$ (change percent)	$P_{T3,T1}$ (change percent)	$P_{T2,T1}$ (change percent)	$P_{T3,T2}$ (change percent)	$P_{T3,T1}$ (change percent)	$P_{T2,T1}$ (change percent)	$P_{T3,T2}$ (change percent)	$P_{T3,T1}$ (change percent)	$P_{T2,T1}$ (change percent)	$P_{T3,T2}$ (change percent)	$P_{T3,T1}$ (change percent)
WC (cm)	0.000↓ (3.1±0.5)	0.003↓ (1.5±0.4)	0.000↓ (4.6±0.5)	0.000↓ (5.4±0.6)	0.010↓ (2.2±0.8)	0.000↓ (7.6±1)	0.000↓ (3.6±0.5)	0.000↓ (1.8±0.4)	0.000↓ (5.5±0.6)	0.000↓ (5.1±0.7)	0.046↓ (1.3±0.6)	0.000↓ (6.4±1)
SBP (mmHg)	0.06↓ (4±9)		0.009↓ (8±11)	0.009↓ (6±11)			0.099↓ (4±10)					
DBP (mmHg)												
FBS (mg/dl)				0.007↑ (9±12)	0.041↓ (6±11)							
TG (mg/dl)		0.009↓ (9±13)	0.05↓ (12±17)	0.001↓ (19±19)	0.088↑ (14±32)					0.084↓ (5±19)		
HDL-C (mg/dl)												
Insulin (μIU/ml)	0.039↑ (31±49)						0.076↓ (10±46)		0.093↓ (9±30)			
HOMA-IR	0.002↑ (35±41)	0.031↓ (29±76)										
AST (U/l)		0.000↓ (30±26)										
ALT (U/l)		0.038↓ (46 ± 73)			0.030↓ (44 ± 60)	0.071↓ (15 ± 9)			0.007↓ (13 ± 62)			
NO_x (μmol/l)	0.028↑ (15±32)		0.024↑ (9±14)				0.028↓ (4±50)				0.055↓ (18±76)	0.017↓ (35±55)

Values are means ± SD. HCD: Hypocaloric Control Diet; Arg: L-arginine; Se: Selenium; T_1: Before intervention; T_2: Three weeks after intervention; T_3: Six weeks after intervention; WC: Waist Circumference; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; FBS: Fasting Blood Sugar; TG: Triglyceride; HDL: High Density Lipoprotein; HOMA – IR: Homeostasis model of insulin resistance; AST: Aspartate Amino Transferase; ALT: Alanine Amino Transferase; NO_x: Nitrite/Nitrate.

arginine + selenium was observed on diastolic blood pressure (DBP), TG, HDL, AST and ALT.

With paired *t*-test or Wilcoxon modeling, the following results were obtained (Table **2**): after 6 wk, all of treatments significantly reduced WC; HCD significantly reduced TG (P=0.05) and SBP (P = 0.009); and HCD+Selenium significantly reduced ALT (P = 0.007).

When Mann-Whitney U test was used, the percent of WC change in 6 weeks in HCD+Arg group was significantly more than HCD (P = 0.008); while, for HCD+Arg+Se group it was marginally (P = 0.067) more than HCD group.

DISCUSSION

In current study, selenium significantly reduced fasting serum concentrations of insulin and HOMA-IR. In animal models and cell-culture studies, selenium showed insulin-like action and accelerated insulin release from isolated pancreatic islets dose dependently. Selenium also increased insulin secretory reserve in selenium deficient animals [9]. In Ozkaya cross-sectional study, serum concentrations of selenium in first-degree relatives of diabetic patients were significantly lower than control group. Also, the amount of HOMA-IR in individuals with selenium concentrations ≥ 80 μg/L was significantly lower than HOMA-IR in individuals with selenium concentrations < 80 μg / L [20]. Despite these findings, in Americans where their selenium intake (60-220 μg / d) is well above the recommended dietary allowance of 55 μg / day, and serum concentrations of selenium is high [21], selenium supplementation did not prevent type 2 diabetes, and increased risk of it [11]. In a representative sample of the Americans, high serum concentration of selenium was positively associated with the prevalence of diabetes [11]. In the Third National Health and Nutrition Examination Survey, subjects in the lowest quintile of serum selenium concentration had a reduced prevalence of diabetes compared with those in the highest quintile [10], and Nutritional Pre-

vention of Cancer trial showed a high risk for diabetes among subjects randomly allocated to receive 200 μg / d of selenium for 7.7 years compared with placebo [12]. The cause for this contradiction can be attributed to the high serum concentration levels of selenium in the US population. Serum selenium concentration levels of the US society even in low quintile are higher than Tabrizi women when this study conducted on them. The average serum selenium level in healthy women in Tabriz city was 76.67 μg / L which is lower than those reported in some other countries. Fifty seven percent of Tabrizi women had serum selenium concentrations under 80 μg / L and serum selenium concentrations did not vary with age and body mass index [13]. It seems that not only higher concentration of serum selenium but its lower concentrations also will impair glucose metabolism and insulin sensitivity. It is therefore that the conflicting results in this area can be attributed to the baseline level of serum selenium of the study population.

By willcoxon analysis HCD + Selenium significantly reduced ALT after 6 week intervention. Zimelanski studied the effect of selenium enriched diet on fat accumulation in liver on vistar rats. In this study, the effect of selenium was two phasic: in the first 12 months of selenium administration liver fat accumulation was reducing; while, after 18 months this effect disappeared and there was no significant difference between intervention and control group [22]. In the cross-sectional study of Navarro-Alarcon, mean serum concentration of selenium in patients with cirrhosis or hepatitis was significantly lower than the control group and there was significant reverse relationship between serum concentration of selenium and γ – glutamil transferase. In this study, the intensity of liver abnormality determined disturbance of serum selenium concentration. In other words when intensity of liver injury increased, serum concentration of selenium significantly reduced [23]. Burk showed that disturbance in serum concentration of selenium in patients with cirrhosis increased glutathione peroxidase activity in serum. This study showed no selenium deficiency in cirrhotic patients [24]; while, Al-bader showed that selenium deficiency created cirrhosis in rats [25]. Although the above mentioned trials are about liver hepathopathies, these findings can be expanded to NAFLD. Selenium as a cofactor of glutathione peroxidase enzyme has an important role in some of biochemical reactions. This enzyme can also prevent initiation and progression of liver injury by oxidants. Probably, the protective effect of selenium occurs *via* its antioxidant role on hydroperoxide ions. Although, low serum concentration of selenium is not the cause for hepathopathy; however, the defect in the protective mechanisms of the body against free radicals may initiate it. Insulin resistance created from central obesity increases lipolysis for providing the caloric needs of the body. Lipolysis increases free fatty acid concentration in serum and fatty acids accumulation in liver [26]. The effects of selenium on reducing ALT concentration may be interpreted by insulin- like action of selenium. Probably, selenium could reduce insulin resistance, adipose tissue lipolysis and fat accumulation in liver.

HCD significantly reduced WC, TG and SBP in 6 weeks, increased FBS after 3 weeks and returned it to basal level after 6 weeks, and had no significant effect on other parameters. In various interventions which participants consumed similar diet, like our study, SBP, TG and WC were reduced significantly but diet had no significant effect on HDL, FBS, insulin and insulin resistance [27]. The findings of this study are consistent with previous studies which used similar composition of macronutrients. The existence of no relationship between HCD and SBP after controlling for WC can indicate the effect of HCD mediated by WC reduction.

In the current study, L-arginine and L-arginine + selenium supplementation for 6 weeks had no significant effect on SBP, DBP, FBS, TG, HDL, insulin and HOMA-IR. In Lucotti study, 8.3 g/d oral L-arginine supplementation in 3 weeks, significantly reduced SBP, DBP, FBS, insulin and HOMA-IR in obese, insulin resistant type 2 diabetic patients but it showed no significant effect on serum TG [3]. In the research by Martina L-arginine intravenous administration (1,200 mg Zentrum, one vial a day) plus *N*-acetylcysteine (600 mg Acetilcisteina, one tablet twice a day) reduced SBP and DBP and increased HDL significantly in hypertensive patients with type 2 diabetes in 6 months, but it did not affect TG [4]. In another study performed by Piatti on the patients with type 2 diabetes L-arginine (9 g / d) in 4 weeks significantly reduced SBP but it had no effect on FBS, insulin and DBP [28]. In study on the aortocoronary bypass patients, L-arginine (6.4 g / d) significantly reduced DBP and FBS in 6 months but it did not affect fat mass, fat free mass, WC, SBP, insulin, TG, and HDL [29]. Inconsistency between the results of the current study and the above mentioned findings can be attributed to the following reasons: 1) low dose of L-arginine in our study; 2) the administration of L-arginine along with *N*-acetylcysteine in Martina study; and 3) differences in baseline characteristics and history of diseases.

In consistent with previous studies, selenium supplementation had no significant effect on WC, SBP, DBP, TG, and HDL [30, 31].

The effects of HCD and L-arginine and / or selenium on WC and some cardiovascular disease risk factors are discussed elsewhere [15, 32].

This is the first time that the simultaneous effects of hypocaloric diet, L-arginine and selenium on metabolic features and insulin resistance have been studied. The major advantage of the current study is its applied statistical methods. Although, each group had 17 cases, with Nested M-ANOVA repeated measurements model, simultaneous analysis was used on 68 cases.

There were several limitations. First, the estimation of body fat with advanced procedures was not possible. Second, serum concentrations of L-arginine and selenium were not measured. Third, the application of a basic hypocaloric diet impacted net effect of treatments.

CONCLUSION

We concluded that selenium supplementation significantly lowered fasting concentrations of serum insulin, HOMA-IR and ALT in females with central obesity; L-arginine significantly reduced WC; whereas, HCD significantly lowered SBP, TG and WC. Long term studies for approving these results are necessary.

CONFILICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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