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Original Article

Synergistic Effect of Nordic Walking Training and Fenugreek Supplementation on Adiponectin and Asprosin in Diabetic Older Adult Women; Simultaneously Using a Multivariate Longitudinal Model: Randomized Clinical Trial

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Abstract

Introduction: This study aimed to evaluate the synergistic effect of Nordic walking (NW) training and fenugreek supplementation (FS) on plasma adiponectin and asprosin concentrations in elderly women with diabetes.

Methods: In a randomized, double-blind, placebo-controlled clinical trial, a total of 48 elderly women with type 2 diabetes (mean age of 66.73 ± 1.52 years) were randomly allocated into six groups of equal size: control group (n = 8), FS group (n = 8), placebo group (n = 8), NW+FS group (n = 8), NW+placebo group (n = 8), and NW group (n = 8). The exercise protocol was NW training, administered on non-consecutive days over eight weeks, at a frequency of three days per week. The NW+FS and FS groups were administered a daily dosage of six capsules, each containing 300 mg of phytophage herbal tablet (equivalent to 220 mg of standardized dried fenugreek extract.)

Results: The synergistic effect of exercise and supplementation significantly reduced FBS by an average of 16.88 mmHg, HOMA-IR by 1.74 units, and asprosin by 0.61 ng/dl compared to the control ($\beta = -16.88$, $P < .001$; $\beta = -2.74$, $P = .009$; and $\beta = -0.61$, $P = .007$, respectively). So, the synergistic effect of exercise and supplementation on FBS, HOMA-IR, and asprosin was 2.42, 1.44, and 3.86, respectively. However, this synergistic effect on insulin was not statistically significant ($\beta = -2.10$, $P < .065$). On the other hand, the combined intervention resulted in a significant increase in adiponectin levels ($\beta = 13.00$, $P < .001$).

Conclusion: Both NW training and FS have beneficial effects on inflammatory and anti-inflammatory factors in older women with diabetes.

Introduction

There is an increase in the ageing population, which is usually accompanied by an increase in body fat and visceral fat until the age of 65. This is often accompanied by diabetes or glucose intolerance.[1] Diabetes mellitus (DM) is a prevalent chronic ailment and the most widespread endocrine disorder globally, encountered in most countries.[2] Type 2 diabetes mellitus (T2DM) is a multifaceted metabolic disorder that results in altered protein, fat, and carbohydrate metabolism. It is distinguished by a gradual decline in β-cell insulin secretion caused by relative insulin insufficiency or diminished tissue responsiveness to insulin.[3] Research indicates that many genes undergo alterations in their expression patterns with advancing age, potentially contributing to the development of comorbidities associated with T2DM.[3, 4] Elevated proinflammatory parameters, decreased levels of anti-inflammatory markers, and increased surrogate insulin resistance are risk factors for the development of diabetes in older women in primary care.[5] Asprosin is responsible for many physiological processes, including glucose metabolism, insulin resistance, lipid profile, and appetite stimulation. Plasma concentrations of asprosin are usually determined during these procedures.[6, 7] In vitro investigations have demonstrated that asprosin may exert an additional proinflammatory function in pancreatic β-cells and skeletal muscle cells.[8] Zhang and colleagues (2019) proposed that elevated asprosin levels in adults diagnosed with T2DM could potentially serve as a risk factor for the pathogenesis of T2DM.[9] Furthermore, a reduction in the secretion of adiponectin and the presence of hyperleptinemia are indicative of metabolic syndrome, which in turn elevates the likelihood of developing T2DM.[10] Herbal supplements may be helpful in treating sarcopenia caused by aging because this condition is associated with osteoarthritis and osteoporosis.[11] Fenugreek (Trigonella

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foenum-graecum) taken as a supplement has been shown to control several hormones in postmenopausal women, causing estradiol, free testosterone, and progesterone levels to rise.[12] Furthermore, research findings suggest that fenugreek seeds may be beneficial in the management of diabetes.[13, 14] Studies have shown that lifestyle changes, such as decreased physical activity and increased obesity, lead to poor health outcomes and in old age, these factors can also cause many problems for the medical staff.[15] Exercise is often prescribed as a treatment to improve health, but many studies link it to better outcomes.[16, 17] Additionally, research has shown that Nordic walking (NW) training offers many benefits for older adults.[18] According to a study by Park et al, combined exercise can improve biochemical factors, anabolic effects, and daily living activities in elderly women who have osteoarthritis and sarcopenia.[19] Inflammatory markers such as asprosin and anti-inflammatory markers such as adiponectin have a role in the pathogenesis of T2DM that is not well understood. This study used multivariate longitudinal models to evaluate the synergistic effect of NW training and FS on plasma adiponectin and asprosin concentrations in diabetic older women.

Materials & Methods

Study Design and Participants

All procedures performed in studies involving human participants were in accordance with the ethical standards of the 1964 Declaration of Helsinki. Also, each subject in the project signed a detailed informed consent form. Several factors were considered to determine the appropriate sample size for the study, including a significance level of $\alpha = 0.05$, a power of $1\text{-}\beta = 0.8$, a synergy effect factor of -5.56 with a standard error of effect of 0.94, and a standard deviation of FBS of 6.05 [20]. Based on these parameters, it was determined that a minimum sample size of six participants per group was necessary. However, to account for a potential dropout rate of 20%, the sample size was increased to eight participants per group. The sample size was calculated using PASS 2023 (NCSS, LLC, Kaysville, Utah, USA, ncss.com/software/pass). In collaboration with the Diabetes Association of Babol City, in the Mazandaran province of Iran, a sample of 48 older women diagnosed with T2DM, with a mean age of 66.73 ± 1.52 years, was selected using convenience sampling. The inclusion criteria for this study were as follows: female gender, age between 65 and 75 years, a confirmed diagnosis of T2DM for a minimum of 10 years with approval from the Diabetes Association, no prior history of heart disease, and no record of physical exercise for at least six months. Exclusion criteria included the following: acute illness (including acute joint disease, weak bones, and fractures), inability to exercise, use of tobacco and alcohol, use of antioxidant supplements, and unwillingness to participate in any study. Upon completion of the informed consent form, the physical activities were conducted following a preliminary health assessment of the subjects by a physician and relevant tests one week prior to the commencement of the procedure. Figure 1 demonstrates the CONSORT diagram for the FS-NW RCT, showing participant flow through the study. Participant numbers at each stage are shown in this figure.

The subjects' height, weight, and body composition were measured during this assessment. Forty-eight hours prior to the commencement of the protocol, blood specimens were collected from the antecubital fossa at 8-9 a.m., by a proficient nurse following an overnight fast of 8-10 hours. The present study employed the Randomization List procedure of NCSS software version 23.0.2 to randomly allocate 48 participants into six groups of equal size: control group (n = 8), FS group (n = 8), placebo group (n = 8), NW+FS group (n = 8), NW+placebo group (n = 8), and NW group (n = 8). Block randomization with equal allocation was utilized for this purpose. To ensure allocation concealment, the randomization list was prepared by an independent researcher not involved in participant recruitment or assessment. Group assignments were placed in sequentially numbered, sealed, opaque envelopes, which were opened only after a participant had been enrolled and baseline measurements were completed.

The maximum heart rate and heart rate reserve were calculated by measuring the resting heart rate before the study, and the exercises started at an intensity equal to 50% of the maximum reserve heart rate. The exercise procedure was also taught to the subjects in the exercise group during the familiarization period. The exercise groups consisted of three groups: the NW+FS group, the NW+placebo group, and the NW group. These groups underwent an 8-week exercise program comprising three sessions per week on non-consecutive days. Each session lasted 60 minutes and was conducted in the morning, typically between 11:00 a.m. and 1:00 p.m. This timing was chosen to ensure that exercise was performed at least two hours after insulin intake. The exercise protocol employed in this study was Nordic walking training.[21]

A fenugreek herbal supplement was prepared as a 300 mg phytophage herbal tablet containing 220 mg of standardized dried extract of the fenugreek plant from Iran Daruk Company. The NW+FS and FS groups received a daily dosage of six capsules, each containing 300 mg of the active ingredient. The participants were instructed to consume two capsules prior to every meal. The placebo and NW+ placebo groups received capsules containing roasted wheat flour as a placebo. Before consuming herbal supplements, the medical personnel ensured that any potential drug interactions were thoroughly evaluated and duly considered. In addition, to control for nutritional intervention factors, a food memory questionnaire was used. Following an 8-week training period under the supervision of a trained coach, post-test assessments were administered under identical conditions to the pre-test, 48 hours after the final training session. The researchers and the subjects were kept unaware (blinded) of the study groups to which the participants belonged. Furthermore, participants were asked to maintain their

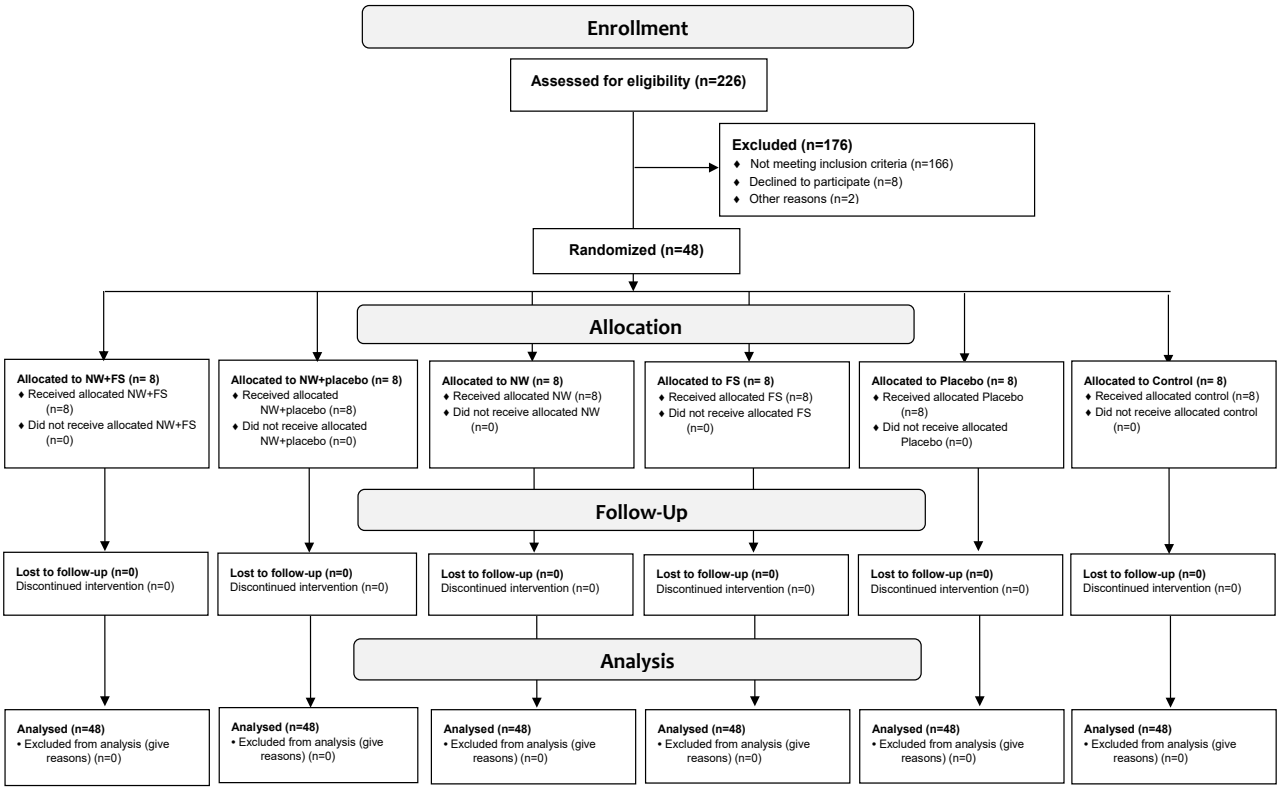

Figure 1. CONSORT diagram for the FS+NW RCT, showing participant flow through the study. Participant numbers at each stage are shown in the figure

dietary and physical activity habits throughout the study. The participants were monitored biweekly for capsule distribution and to assess any potential adverse effects or sports-related injuries.

Training Intervention

The present investigation employed the NW training protocol, administered on non-consecutive days for eight weeks with a frequency of three days per week. One week prior to the commencement of the training protocol, the subjects underwent two training sessions under the guidance of an experienced trainer. These sessions were designed to provide comprehensive instruction on the proper handstick training techniques. A heart rate monitor was affixed to the subjects' bodies during the training sessions to regulate training intensity. Additionally, the subjects were acquainted with the Polar heart rate monitor and watch used to monitor and regulate training intensity. Each training session lasted 60 minutes, with an intensity ranging from 50% to 70% of VO2 max. The NW training protocol entails utilizing more muscles in the body and a higher degree of intensity than conventional walking. In most previous research, exercise sessions were typically 30 minutes long, and the intensity of the exercises was commonly determined by step count. In this study, the exercise regimen was shortened to eight weeks, and the frequency of training sessions was set to three times per week to achieve improved outcomes and refine the protocols utilized in prior investigations. However, to ensure that the total training volume in eight weeks was comparable to that of most research protocols, each training session was extended to 60 minutes to match the total training volume of eight weeks with the volume of regimens in most studies[22] Exercise intensity was calculated using a Polar heart rate monitor, and based on previous research stating that the best intensity to improve insulin resistance is 50-70% VO2 max, the workout routine started at an intensity of 50% VO2 max and increasedby week four. Based on the overload principle and research, the intensity reached 70% of VO2 max by week eight.

Assessments

All assessments were recorded at the start of the study and at the end of the intervention.

Anthropometric and Dietary Assessments

Weight was measured to the nearest 100 g using a digital scale while individuals were dressed in light clothing without shoes. Height was measured to the nearest 0.5 cm when standing without shoes using a tape measure. BMI was calculated as weight (kg) divided by the square of height (m^2). A 24-hour dietary record (two business days and one weekend) was completed by subjects at the start and end of the study to assess dietary intake.

Clinical and Laboratory Assessments

In order to assess the levels of adiponectin, asprosin, insulin, and glucose in the participants' bloodstream, a 5cc blood sample was extracted from the brachial vein following an overnight fast of 8 to 10 hours. The samples were collected into tubes containing EDTA and analyzed before and after the training protocol. Blood samples were centrifuged at 3000 rpm for 12 minutes and stored

at -35°C to measure variables. Insulin resistance levels are calculated using the following formula:

$$HOMA-IR = \left(\text{glucose}_{\text{mmol/L}} \times \text{insulin}_{\text{mIU/mL}}\right) / 22.5$$

Plasma concentrations of adiponectin and asprosin were measured using commercially available human-specific ELISA kits (ZellBio GmbH, Germany). For adiponectin, kit Cat. No. ZB-11550C-H9648, Lot No. AD34213, with a detection limit of 0.68 ng/mL, Intra-assay CV%: 3.2%, Inter-assay CV%: 5.6% were used. For asprosin, kit Cat. No. ZB-15879H, Lot No. ASP-20230522, with a detection limit of 0.73 ng/mL, Intra-assay CV%: 2.5%, Inter-assay CV%: 4.8%, was applied. All assays were performed strictly according to the manufacturer's instructions to ensure reproducibility and comparability with other studies.

Plasma glucose was measured using an enzymatic colorimetric method, specifically the Glucose Oxidase-Amino Antipyrine (GOD-PAP) technique, as provided by Pars Azmoun in Tehran, Iran.

Statistical Analysis

Quantitative variables were summarized using statistical indices such as the mean and standard deviation for descriptive purposes. In order to facilitate analytical procedures, the initial step involved the utilization of the paired sample t-test on the scale of BMI, insulin, FBS, adiponectin, and asprosin for each group (control, FS, placebo, NW+FS, NW+placebo, and NW) at two distinct time points (baseline and eight weeks later), independently. Moreover, because of the nature of repeated measures that are often collected through multiple correlated responses, in the first step, the correlation between variables was evaluated and displayed using a repeated measure correlation (*rmcorr*) heatmap for data that involved subjects with multiple measurements. The correlation heatmap is a graphical representation in which a row and a column depict each variable, and the cells within the matrix display the correlation between them. The color intensity in each cell indicates the magnitude and direction of the correlation, with darker hues indicating stronger correlations. In the second step, multivariate longitudinal modeling and generalized estimating equations (GEE) methodology with the correlation structure AR (1) were used for valid statistical inferences.[20] With this model, in addition to the within-subject association, the association of multivariate responses at a specific time point was also considered to evaluate the synergistic effect of NW training and FS on repeated multivariate measurements of BMI, FBS, adiponectin, and asprosin while controlling for participant age.[23] The synergistic effect is then defined as: synergistic effect $= \beta_{NW(yes)FS(yes)} / (\beta_{NW(yes)FS(no)} \times \beta_{NW(no)SF(yes)})$. It is the ratio of the observed β-parameter estimate of the multivariate model for NW training and FS to the predicted β assuming independent effects of each factor.[24] All analyses were performed in R-3.4.1.[25] A P-value less than 0.05 was considered statistically significant. Because multiple comparisons were performed across several groups and outcomes, the risk of type I error inflation was considered. However, given the relatively small sample size and the exploratory nature of the study, no formal multiplicity adjustment (e.g., Bonferroni, Holm–Bonferroni, or false discovery rate) was applied. Instead, effect sizes and confidence intervals were reported alongside p-values, and results are interpreted with caution. This limitation has been explicitly acknowledged in the revised *Discussion*.

Results

Table 1 displays descriptive statistics of BMI, WHR, Protein, Mineral, Water, and Fat percentage and Table 2 displays descriptive statistics of FBS, Insulin, HOMA-IR, adiponectin, and asprosin at two distinct time points (baseline and eight weeks later) for each group. The ANCOVA-based comparison of the values obtained after eight weeks in six groups revealed significant differences for FBS, Insulin, HOMA-IR, adiponectin, and asprosin after adjusting for age and baseline ($P < 0.05$).

Also, for a better view of the comparisons, the mean (± SD) values of plasma levels of insulin (A), HOMA-IR (B), plasma levels of adiponectin (C), and plasma levels of asprosin (D) in control and treated groups at two distinct times are given in Figure 2. As shown in Figures 2A, 2B, and 2C, the NW+FS group exhibited the most significant disparity in before-after levels of insulin, adiponectin, and asprosin. Notably, the NW+ Placebo and NW groups also differed significantly in their pre-test and post-test results. As illustrated in Figure 2B, the differences between the before-after results in the three groups, NW+FS, NW+ Placebo, and NW, were nearly equally noteworthy. Nonetheless, no statistically significant disparity was observed in the FS, placebo, and control groups across all variables before and after the intervention.

A heatmap plot was employed to identify patterns and correlations among multiple variables in which subjects had repeated measures (Figure 3). This plot depicted values for *rmcorr* across two axis variables as a grid of colored squares. Darker colors denote stronger correlations, whereas lighter colors denote weaker ones. Warm colors (red) represent positive relationships (when a particular factor rises, the other factor tends to rise). Blue represents negative correlations (when a particular factor rises, the other factor tends to decline). Based on this figure, the correlations between adiponectin and other variables were negative, while positive correlations were observed among the remaining variables. Also, almost all the *rmcorr* coefficients between variables were strong and statistically significant ($P < 0.001$).

Therefore, due to the significant relationship between the variables, multivariate analysis was used to evaluate the effect of NW training and FS. Table 3 presents the results of a multivariate longitudinal model that incorporates an AR (1) correlation structure. According to Table 2, the synergistic effect of exercise and supplementation significantly reduced FBS by an average of 16.88 mg/

Table 1. Comparing mean BMI, WHR, Protein, Mineral, Water, and Fat % at two time points in six groups

Variable	Group	Baseline (mean ± SD)	8 weeks later (mean ± SD)	*P*-value*	*P*-value¥
BMI (kg/m²)	NW£+FS€	24.60 ± 2.47	24.52 ± 2.42	0.621	0.256
	NW	23.20 ± 2.77	23.09 ± 2.81	0.448	
	NW+placebo	22.95 ± 2.84	22.59 ± 2.92	0.107	
	FS	23.64 ± 2.2	23.63 ± 2.99	0.879	
	Placibo	24.28 ± 3.23	24.35 ± 3.04	0.516	
	Control	21.40 ± 1.45	21.53 ± 1.61	0.306	
WHR	NW+FS	0.961 ± 0.05	0.944 ± 0.04	0.567	0.366
	NW	0.963 ± 0.03	0.944 ± 0.02	0.612	
	NW+placebo	0.965 ± 0.04	0.958 ± 0.03	0.057	
	FS	0.964 ± 0.03	0.968 ± 0.03	0.565	
	Placibo	0.958 ± 0.04	0.955 ± 0.04	0.052	
	Control	0.964 ± 0.05	0.976 ± 0.05	0.054	
Protein%	NW+FS	9.83 ± 0.82	10.36 ± 1.19	0.205	0.150
	NW	9.74 ± 0.92	10.11 ± 1.03	0.010	
	NW+placebo	9.74 ± 0.61	10.16 ± 0.72	0.133	
	FS	9.83 ± 0.67	9.98 ± 0.73	0.090	
	Placibo	9.65 ± 0.93	9.94 ± 1.22	0.164	
	Control	9.78 ± 0.86	9.91 ± 1.04	0.329	
Mineral%	NW+FS	3.18 ± 0.56	3.26 ± 0.61	0.037	0.980
	NW	3.23 ± 0.37	3.23 ± 0.40	0.860	
	NW+placebo	3.32 ± 0.25	3.36 ± 0.47	0.679	
	FS	3.27 ± 0.37	3.30 ± 0.38	0.106	
	Placibo	3.36 ± 0.30	3.32 ± 0.52	0.608	
	Control	3.36 ± 0.55	3.41 ± 0.61	0.063	
Total water%	NW+FS	35.63 ± 5.31	35.24 ± 5.49	0.070	0.963
	NW	36.45 ± 3.37	37.88 ± 2.64	0.063	
	NW+placebo	36.44 ± 8.48	38.54 ± 7.74	0.275	
	FS	36.64 ± 2.26	37.44 ± 3.87	0.210	
	Placibo	36.03 ± 3.54	37.16 ± 3.87	0.106	
	Control	36.40 ± 5.17	36.09 ± 5.40	0.191	
Fat%	NW+FS	45.23 ± 2.93	43.58 ± 2.85	0.030	0.759
	NW	47.68 ± 6.48	45.57 ± 7.92	0.040	
	NW+placebo	45.44 ± 4.05	44.86 ± 4.89	0.686	
	FS	45.60 ± 6.98	46.38 ± 5.32	0.411	
	Placibo	47.99 ± 8.19	47.99 ± 7.42	0.998	
	Control	45.05 ± 6.04	46.70 ± 6.26	0.431	

£ NW: Nordic walking; € FS: fenugreek supplementation; *Paired sample t-Test result; ¥ANCOVA result for "8 weeks later" values by adjusting for age and baseline values

dL, HOMA-IR by 1.74 units, and asprosin by 0.61 ng/dl compared to the control (β = -16.88, $P < .001$; β = -2.74, $P = .009$; and β = -0.61, $P = .007$, respectively). So, the synergistic effect of exercise and supplementation on FBS, HOMA-IR, and asprosin was 2.42, 1.44, and 3.86, respectively. However, this synergistic effect on insulin was not statistically significant (β = -2.10, $P = .065$). On the other hand, the combined intervention resulted in a significant increase in adiponectin levels by 13 ng/dl (β = 13.00, $P < .001$). NW training alone reduced asprosin (β = -0.51, $P = .003$) and HOMA-IR (β = -1.41, $P = .024$), but FS alone had no significant effect on any variable.

Discussion

The results indicated that NW training combined with FS significantly increased adiponectin and decreased asprosin, FBS, and HOMA-IR (Table 3). This change is better than exercise and FS alone. On the other hand, the results indicated that NW training led to a reduction in asprosin and HOMA-IR. However, the administration of FS did not yield any significant effects on the variables mentioned above. Also, the synergistic effect of exercise and supplementation on asprosin was the highest. Asprosin exerts a peripheral effect by directly impacting insulin signaling, leading to heightened insulin resistance,

Table 2. Comparing mean BMI, FBS, insulin, HOMA-IR, adiponectin, and asprosin at two time points in six groups

Variable	Group	Baseline (mean ± SD)	8 weeks later (mean ± SD)	*P*-value*	*P*-value¥
FBS (mg/dl)	NW+FS	124.63 ± 8.88	97.38 ± 11.02	<0.001	<0.001
	NW	124.13 ± 9.76	104.38 ± 15.52	0.021	
	NW+placebo	124.13 ± 9.76	104.38 ± 15.57	0.021	
	FS	123.25 ± 14.47	98.38 ± 8.68	0.064	
	Placibo	124.25 ± 14.43	126.13 ± 17.38	0.020	
	Control	124.50 ± 17.06	128.62 ± 18.41	0.049	
Insulin (ng/dl)	NW+FS	25.40 ± 1.78	22.15 ± 2.61	0.001	0.048
	NW	25.25 ± 2.92	22.39 ± 3.77	0.005	
	NW+placebo	25.37 ± 2.94	22.51 ± 3.78	0.005	
	FS	25.26 ± 5.02	23.86 ± 3.45	0.101	
	Placibo	25.42 ± 3.28	26.67 ± 3.54	0.164	
	Control	25.34 ± 4.90	26.05 ± 4.04	0.335	
HOMA-IR	NW+FS	8.13 ± 1.82	5.57 ± 0.53	0.003	0.002
	NW	8.14 ± 1.12	6.09 ± 1.55	0.001	
	NW+placebo	8.27 ± 1.02	6.22 ± 1.43	0.001	
	FS	7.71 ± 2.91	7.41 ± 2.38	0.451	
	Placibo	8.44 ± 2.22	9.28 ± 2.21	0.077	
	Control	8.33 ± 2.60	8.31 ± 2.65	0951	
Adiponectin (ng/dl)	NW+FS	41.95 ± 8.08	59.49 ± 11.21	0.005	0.001
	NW	38.19 ± 6.74	48.11 ± 8.32	0.006	
	NW+placebo	36.44 ± 8.48	45.86 ± 11.39	0.010	
	FS	44.76 ± 13.74	46.31 ± 8.44	0.534	
	Placibo	36.65 ± 9.29	34.40 ± 9.30	0.140	
	Control	38.16 ± 13.68	41.66 ± 12.69	0.215	
Asprosin (ng/dl)	NW+FS	3.29 ± 0.21	2.22 ± 0.87	0.005	0.001
	NW	3.21 ± 0.44	2.45 ± 0.44	0.002	
	NW+placebo	3.27 ± 0.45	2.50 ± 0.44	0.002	
	FS	3.14 ± 0.54	2.96 ± 0.66	0.499	
	Placibo	3.39 ± 0.58	3.39 ± 0.58	0.363	
	Control	3.29 ± 0.72	3.41 ± 0.74	0.351	

£ NW: Nordic walking; € FS: fenugreek supplementation; *Paired sample t-Test result; ¥ANCOVA result for "8 weeks later" values by adjusting for age and baseline values

inflammation, and endoplasmic reticulum stress. Elevated levels of asprosin in a pathological context may serve as a promising biomarker for early changes in glucose metabolism in individuals with obesity and diabetes [8]. In this study, and as expected, NW training and FS reduced the circulating concentrations of asprosin in T2DM patients.[26, 27] Kantorowicz et al showed that an 8-week training program using NW at an intensity corresponding to the FATmax reduced the concentration of asprosin in the bloodstream and decreased visceral adiposity in young women with metabolic disorders.[28]

Furthermore, it has been demonstrated that asprosin induces inflammation, dysfunction of islet β-cells, and apoptosis through the activation of the toll-like receptor 4 (TLR4) expression and JNK phosphorylation in a manner dependent upon the dosage administered.[29] In addition, asprosin has been shown to activate inflammatory/stress-inducing pathways in the endoplasmic reticulum (ER) related to protein kinase C-δ (PKCδ) in skeletal muscle, resulting in decreased sensitivity to insulin.[30] Asprosin stimulates β-cell apoptosis by increasing the expression of caspase3 and inhibiting β-cell autophagy via disrupting the AMP-activated protein kinase (AMPK)- the mechanistic target of rapamycin (mTOR) pathway.[31] In the present study, the changes were insignificant regarding insulin and BMI. Based on Figure 2, the correlations between adiponectin and other variables were negative, while positive correlations were observed among the remaining variables. Also, almost all the rmcorr coefficients between variables were strong and statistically significant. Hong et al reported that asprosin exhibited a positive and independent correlation with HOMA-IR in the context of multiple linear regression.

Furthermore, binary logistic regression analysis demonstrated that asprosin was positively and independently associated with the incidence of both metabolic syndrome and IR.[32] Summarized scientific data on the potential health benefits of fenugreek include

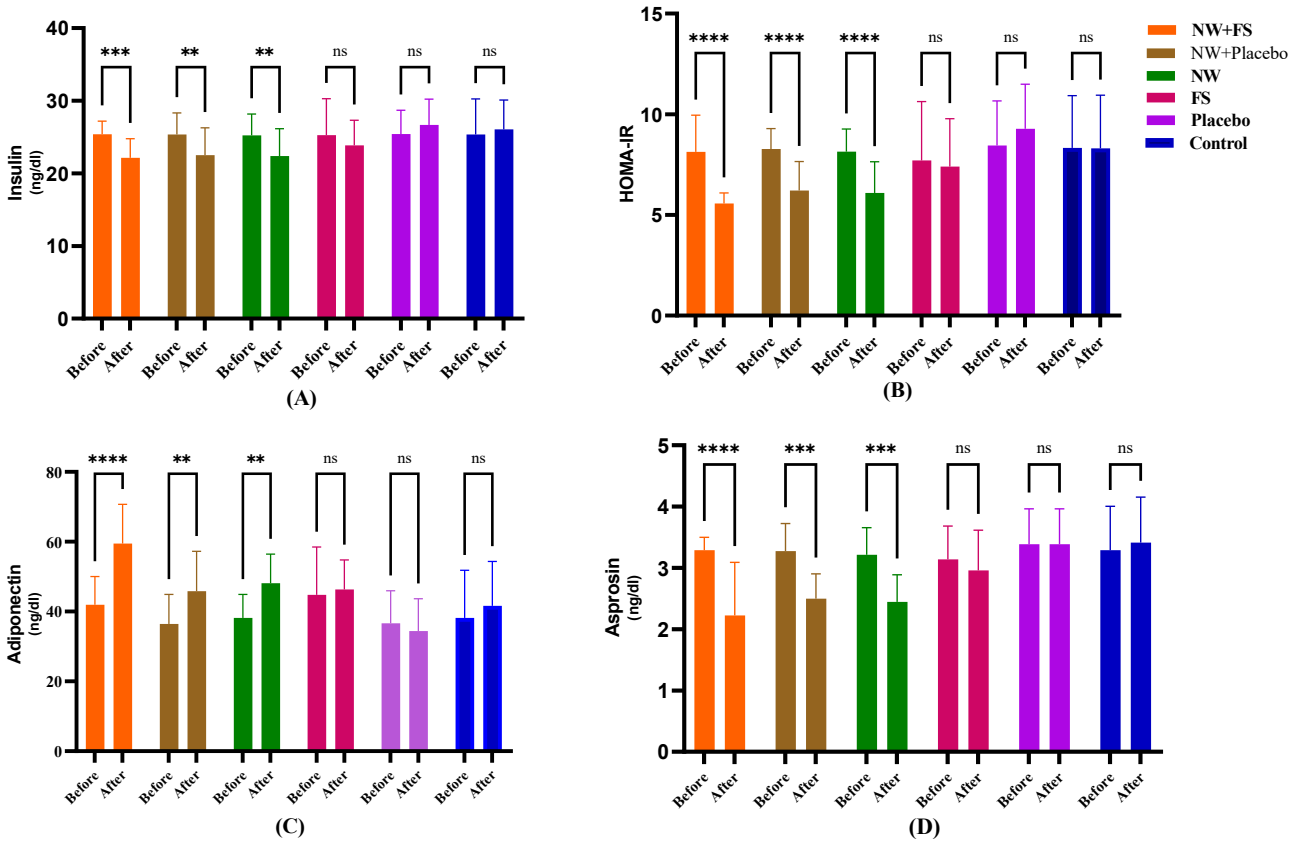

Figure 2. Plasma levels of Insulin (A); HOMA-IR (B); Plasma levels of Adiponectin (C); and Plasma levels of Asprosin (D); in control and treated groups at two distinct time points, namely the baseline (before treatment) and 8 weeks later (after treatment). Abbreviations: NW: Nordic walking training; FS: fenugreek supplementation. A paired t-test was utilized to conduct pairwise before-after comparisons within each group. The P-value results, denoted by asterisks and classified according to their significance level, are presented as follows.: 0.1234 (ns-not significant), 0.0332(*), 0.0021(**), 0.0002 (***), and < 0.0001 (****)

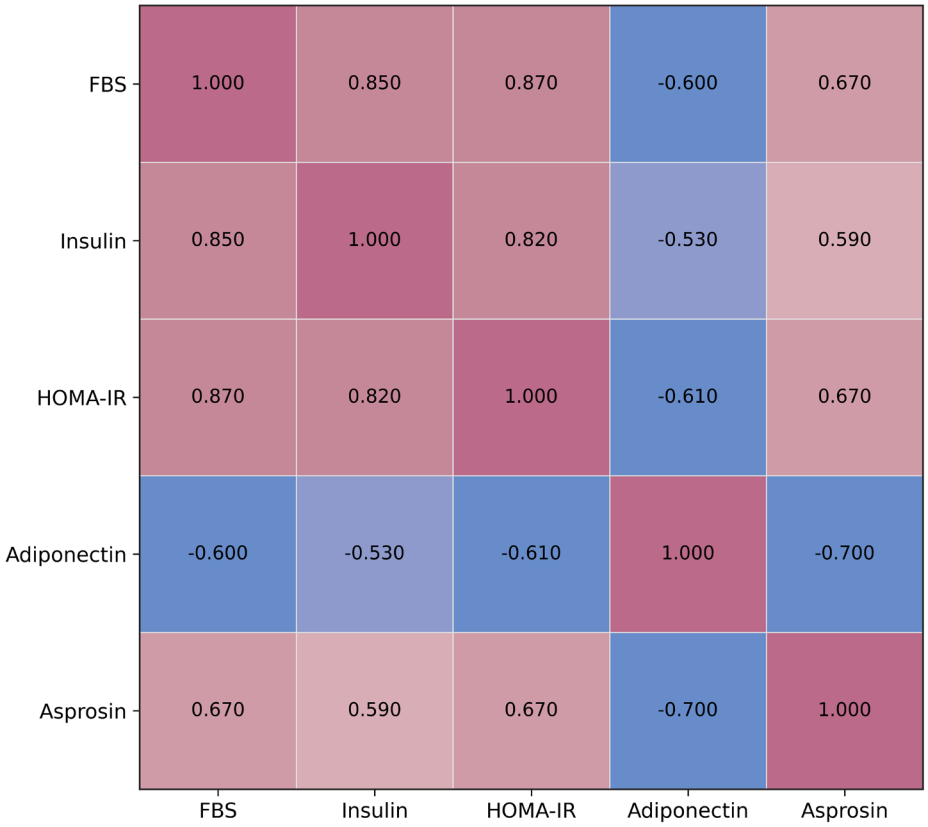

Figure 3. Heatmap of, FBS, Insulin, HOMA-IR, adiponectin and Asprosin using repeated measure correlations. Darker colors indicate stronger correlations, while lighter colors indicate weaker correlations. Positive correlations (when one variable increases, the other variable tends to increase) are represented by warm color (red). Negative correlations (when one variable increases, the other variable tends to decrease) are represented by cool color (blue)

Table 3. Results of multivariate longitudinal model with AR (1) correlation structure

DV[€]	Factor	Category	β[£]	95% CI	*P*
FBS (mg/dl)	Exercise*supplement	Yes*Yes	-16.88	-25.93,-7.82	<0.001
		Yes*No	-2.24	-12.88,8.41	0.680
		No*Yes	-3.11	-16.58, 10.35	0.650
		No*No	Ref. Category		
	Exercise	Yes	-13.63	-22.11, -5.14	0.002
	Supplement	Yes	-6.38	-17.95, 5.20	0.280
		No	Ref. Category		
INSULIN (ng/dl)	Exercise*supplement	Yes*Yes	-2.10	-4.33,0.13	0.065
		Yes*No	-1.99	-4.29,0.31	0.090
		No*Yes	-1.31	-4.54, 1.93	0.428
		No*No	Ref. Category		
	Exercise	Yes	-1.59	-3.44, 0.25	0.091
	Supplement	Yes	-0.71	-2.62, 1.21	0.469
		No	Ref. Category		
HOMA-IR	Exercise*supplement	Yes*Yes	-2.74	-5.04,-0.44	0.009
		Yes*No	-1.41	-2.63,-0.18	0.024
		No*Yes	-1.33	-3.04, 0.99	0.317
		No*No	Ref. Category		
	Exercise	Yes	-1.18	-2.20, -0.15	0.024
	Supplement	Yes	-0.68	-1.79, 0.43	0.228
		No	Ref. Category		
Adiponectin (ng/dl)	Exercise*supplement	Yes*Yes	13.00	5.85,20.15	<0.001
		Yes*No	4.43	-1.95,10.81	0.174
		No*Yes	7.82	-0.94, 16.58	0.080
		No*No	Ref. Category		
	Exercise	Yes	4.68	-0.45, 9.81	0.074
	Supplement	Yes	8.19	2.83, 13.58	0.003
		No	Ref. Category		
Asprosin (ng/dl)	Exercise*supplement	Yes*Yes	-0.61	-1.05,-0.17	0.007
		Yes*No	-0.51	-0.85,-0.17	0.003
		No*Yes	-0.32	-0.76, 0.12	0.150
		No*No	Ref. Category		
	Exercise	Yes	-0.44	-0.71, -0.16	0.002
	Supplement	Yes	-0.21	-0.50, -0.08	0.152
		No	Ref. Category		

[€]Dependent variable; [£] Parameter estimate; [¥] Confidencie Interval

blood sugar management in T2DM, cholesterol-lowering effects, effects on growth hormone leading to metabolic syndrome regulation, anti-inflammatory and anti-cancer effects, and improved milk production in new mothers.[14] Consistent with the findings of this study, Rafraf et al demonstrated that fenugreek seed consumption significantly increased serum adiponectin levels in individuals with T2DM. In addition, fenugreek seed intake also helped reduce fasting blood sugar, insulin, the homeostatic model assessment of insulin resistance, and total cholesterol levels.[33] Of course, attention to the number of meals in a day, meat consumption, and fruit serving also could be helpful.[34] It was hypothesized that fenugreek positively affects insulin resistance and obesity-related inflammation due to its potential to inhibit the iRhom2/TACE axis, facilitating the release of TNF-α from adipocytes.[35] Based on the findings of this investigation, the combination of NW training and FS was associated with increases in adiponectin and decreases in asprosin. However, given the very small sample size (n = 8 per group, these findings should be considered preliminary. For example, a reduction of 0.61 ng/mL in asprosin was observed, but the biological or clinical relevance of this magnitude of change remains uncertain. Therefore, the observed effects suggest a potential synergistic interaction, yet definitive conclusions about synergy or clinical impact cannot be drawn. Adiponectin has been identified as a prognosticator of bone mineral density in

premenopausal women of middle age.[36] Exercise-induced weight loss significantly reduced abnormal bone growth in osteoporotic rats and restored bone homeostasis[37], suggesting that increased adiponectin levels may have therapeutic benefits in osteoporosis.[38] Of course, in this study, BMI did not change significantly, and it is possible that because of NW training, it did not affect weight much, which is also natural. Given the current condition of the elderly population, strength training or concurrent exercise may serve as a viable intervention. Due to being old and the low levels of public knowledge, risk factors, and therapy approaches, increasing public education is necessary for patients and personnel.[39] To comprehensively investigate the impact of various forms of exercise, varying dosages of fenugreek, and other herbal remedies on inflammatory factors, such as asprosin, and their relationship with elderly individuals and those with diabetes, it is imperative that long-term and in-depth research studies be conducted in the future.

Limitations

findings. First, the relatively small sample size (n = 8 per group) reduces the statistical power and may limit the detection of subtle effects. Second, the study population was demographically homogeneous, consisting only of older Iranian women, which restricts the generalizability of the results to other populations, including men, younger individuals, and people from different ethnic or regional backgrounds. Third, all participants were recruited from a single study site, which further limits external validity. In addition, the short duration of the intervention and the absence of a follow-up period prevent conclusions about the long-term sustainability or durability of the observed effects. Future studies with larger, more diverse populations, multicenter designs, and extended follow-up are needed to confirm and expand upon these findings.

Conclusion

In summary, both NW training and FS have beneficial effects on inflammatory and anti-inflammatory factors in older women with diabetes. The synergistic effect of the two treatments increases adiponectin while decreasing asprosin, FBS, and HOMA-IR more than either treatment alone. This is the first demonstration of the synergistic effect of the combination of NW training and a dietary supplement (FS) on inflammatory and anti-inflammatory factors in older women with diabetes.

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Competing Interests

The authors declare no conflict of interest.

Ethical Approval

This study was approved by the institutional ethics committee of tabriz university of medical sciences under the code IR.TBZMED.REC.1402.378. A randomized, double-blind, placebo-controlled clinical trial was conducted from december 2023 to march 2024 in the mazandaran province of iran (registered on irct.behdasht.gov.ir (clinical trial registry number: IRCT20231110060008N1||2023.11.22)). All procedures performed in studies involving human participants were in accordance with the ethical standards of the 1964 helsinki declaration.

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