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Original Research

Effectiveness of Genistein Supplementation on Metabolic Factors and Antioxidant Status in Postmenopausal Women With Type 2 Diabetes Mellitus

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Key Messages

- Genistein supplementation for 12 weeks was assessed in postmenopausal women with type 2 diabetes mellitus.
- Genistein supplementation reduced fasting blood glucose, glycated hemoglobin (HbA1C) and serum triglyceride levels, as well as improved antioxidant status.
- Genistein supplementation may be useful in controlling metabolic status and oxidative stress in postmenopausal women.

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ABSTRACT

Objectives: The risk of type 2 diabetes mellitus (T2DM) increases in women after menopause. Genistein is known to modulate metabolic pathways. The aim of this study was to investigate the effects of genistein supplementation on metabolic parameters, oxidative stress and obesity values in postmenopausal women with T2DM.
Methods: This randomized, double-blind, placebo-controlled clinical trial was conducted on 54 postmenopausal women 47 to 69 years of age with T2DM. The genistein group (n=28) was given 2 genistein capsules daily for 12 weeks. Each capsule contained 54 mg genistein. The placebo group (n=26) received 2 placebo capsules daily for the same period. Fasting blood samples, anthropometric measurements, dietary intakes and physical activity levels of subjects were collected at baseline and at the end of the trial. Data were analyzed by independent *t* test, paired *t* test and analysis of covariance.
Results: Genistein supplementation significantly reduced serum levels of fasting blood glucose (FBS), glycated hemoglobin (A1C), serum triglyceride (TG) and malondialdehyde (MDA) and increased total antioxidant capacity (TAC) compared with the placebo group at the end of the study ($p<0.05$ for all). Serum high-density lipoprotein cholesterol and quantitative insulin sensitivity check index significantly increased within the genistein group. Changes in anthropometric indexes and other variables were not significant in any of the groups.
Conclusions: Genistein administration improved FBS, A1C, serum TG, TAC and MDA in postmenopausal women with T2DM and may be useful in the control of metabolic status and oxidative stress in these subjects.

Mots clés :
statut antioxydant
génistéine
facteurs métaboliques
obésité
femmes postménopausées
diabète sucré de type 2

RÉSUMÉ

Objectifs : Le risque de diabète sucré de type 2 (DST2) augmente chez les femmes après la ménopause. Il est connu que la génistéine module les voies métaboliques. L'objectif de la présente étude était

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d'examiner les effets de la supplémentation en génistéine sur les paramètres métaboliques, les valeurs en matière de stress oxydatif et d'obésité chez les femmes postménopausées atteintes du DST2.
Méthodes : L'étude clinique à répartition aléatoire et à double insu, contre placebo, a été réalisée chez 54 femmes postménopausées atteintes du DST2 qui étaient âgées de 47 à 69 ans. Les femmes du groupe de la génistéine (n = 28) ont reçu quotidiennement 2 capsules de génistéine durant 12 semaines. Chaque capsule contenait 54 mg de génistéine. Les femmes du groupe du placebo (n = 26) ont reçu quotidiennement 2 capsules d'un placebo durant la même période. Les échantillons de sang à jeun, les mesures anthropométriques, les apports alimentaires et les niveaux d'activité physique des sujettes ont été recueillis au début et à la fin de l'étude. Les données ont été analysées au moyen du test *t* pour échantillons indépendants, du test *t* pour échantillons appariés et de l'analyse de covariance.
Résultats : Le groupe de supplémentation en génistéine, et non le groupe du placebo, a montré une réduction significative des concentrations sériques de la glycémie à jeun (GJ), de l'hémoglobine glyquée (A1c), des triglycérides (TG) et du malondialdéhyde (MDA), et une augmentation de la capacité antioxydante totale (CAT) à la fin de l'étude ($p < 0,05$ pour tous). Les concentrations sériques de cholestérol à lipoprotéines de haute densité et l'indice QUICKI (de l'anglais, *quantitative insulin sensitivity check index*) ont augmenté de façon significative dans le groupe de la génistéine. Aucun groupe n'a montré des changements significatifs dans les indices anthropométriques et les autres variables.
Conclusions : L'administration de génistéine a permis d'améliorer la GJ, l'A1c, les TG sériques, la CAT et le MDA chez les femmes postménopausées atteintes du DST2, et peut être utile à la régulation des voies métaboliques et du stress oxydatif chez ces sujettes.

Introduction

Type 2 diabetes mellitus (T2DM) is one of the most common and serious metabolic disorders, characterized by elevated blood glucose levels and insulin resistance (1). The global prevalence of diabetes was 382 million people in 2013, and it is estimated to reach 592 million by 2035 (2). Diabetes-related complications including peripheral neuropathy, nephropathy, retinopathy and cardiovascular disease have a high health burden (3).

Some investigations have reported higher occurrence of metabolic diseases and reduced insulin sensitivity in postmenopausal women compared with premenopausal women (4,5). Estrogen loss, elevated free testosterone, weight gain and changes in body composition during menopausal transition promote insulin resistance in postmenopausal women (6).

Estrogen plays an important role in the amelioration of insulin resistance and lipid metabolism in the liver and muscles (7). Several studies have demonstrated beneficial effects of hormonal replacement therapy on improving glycemic control and insulin resistance in postmenopausal women with T2DM (8,9). However, hormonal replacement therapy has been associated with an increased risk of breast and endometrial cancer (10). Recent evidence strongly suggests that consumption of foods containing high phytoestrogens, such as soybeans, plays an important role in protecting against obesity, T2DM and cardiovascular disease without adverse effects (11).

Phytoestrogens are a group of biological compounds that act through the estrogen receptor (ER) because of structural similarity to estrogen (12). The preeminent studied subgroup of phytoestrogens is isoflavones, which is largely found in soybeans; it contains about 1.2 and 4.2 mg isoflavone/g (13). The 3 major soy isoflavones are genistein, diadzien and glycitein in generally a concentration ratio of 1:1:0.2 (14).

Genistein (4′, 5, 7-trihydroxyisoflavone) is the most abundant soy isoflavone, which shows a lower estrogenic potency than 17-beta-estradiol. The binding affinity of genistein for ER-alpha and ER-β is 4% and 87%, respectively (12). Therefore, it may act as a natural selective ER modulator for these receptors (15). Genistein also has other biological actions, including inhibition of protein-tyrosine kinase activity and antioxidant and anti-inflammatory characteristics (16,17). A number of animal and human studies have proposed its protective effects against metabolic disturbances (18–21). Gupta et al (18) reported that genistein treatment decreased blood glucose and glycated hemoglobin (A1C) levels and ameliorated glucose intolerance in a diabetes rat model. Squadrito et al (21) showed that genistein supplementation improved insulin resistance and lipid profiles in postmenopausal subjects with metabolic syndrome. The positive effects of genistein on fasting glucose, insulin, insulin resistance and anthropometric parameters have also been suggested in patients with nonalcoholic fatty liver disease (20) and healthy postmenopausal women (19).

Oxidative stress is an important outcome of chronic hyperglycemia and plays a critical role in the development of insulin resistance in subjects with diabetes (22). Some studies indicated that genistein increases gene expression of antioxidant enzymes (23). Wang et al (24) demonstrated that treatment with genistein in low-density lipoprotein receptor knockout mice increased total antioxidant capacity (TAC) and superoxide dismutase activity, and decreased serum malondialdehyde (MDA) levels.

Based on results from human and animal studies, genistein may exert an effective role in metabolic status. However, no study is available about the possible effects of genistein in postmenopausal women with T2DM. Therefore, this study was carried out to investigate the effects of genistein supplementation on glycemic indexes, serum lipid profiles, oxidative stress and obesity values in postmenopausal women with T2DM.

Methods

This randomized, double-blind, placebo-controlled clinical trial was conducted on 60 postmenopausal women with T2DM. The study protocol was approved by the Ethics Committee of the Tabriz University of Medical Sciences, and registered in the Iranian Registry of Clinical Trials (http://www.irct.ir/) with IRCT registration number IRCT201611033664N18. The study was also conducted in accordance with the guidelines of the Declaration of Helsinki principles.

Participants

A total of 143 postmenopausal women with T2DM were screened from the endocrinology clinic, Dr. Gholipour Hospital in Boukan, Iran, from April 2017 to November 2017. Finally, 60 patients 47 to 69 years of age were enrolled with the following inclusion criteria: postmenopausal women (menstrual cessation for at least 12 months prior to intervention, follicle-stimulating hormone level

≥50 IU/L and serum 17β-estradiol level ≤100 pmol/L) and diagnosed with T2DM (for at least 6 months before the beginning of the study). The exclusion criteria were as follows: insulin injection; use of estrogen or any hormonal replacement therapy; having surgery-induced menopause; suffering from cardiovascular disease, chronic renal or hepatic failure, thyroid disorders, breast disease or family history of breast cancer; smoking; alcohol consumption; and use of nutritional supplements 3 months before the intervention. In addition, subjects taking medicines that affect glucose metabolism, such as nonsteroidal anti-inflammatory drugs or steroids, corticosteroids (prednisone, prednisolone and hydrocortisone) and second-generation antipsychotics (olanzapine and clozapine), in the last 3 months were also excluded. Informed consent was obtained from all individual participants included in the study.

The sample size was determined based on change in homeostasis model assessment of insulin resistance (HOMA-IR), which was obtained from the Squadrito et al study (21). By considering a confidence level of 95%, a power of 80%, and a 2-tailed test, the sample size was computed to be 25 per group. To provide accommodation for the anticipated dropout of 20%, the sample size was increased to 30 patients in each group.

Study design

The selected patients were randomly assigned to the experimental and control groups by using a block randomization procedure while matching subjects based on body mass index (BMI) and age. The random sequences were generated by random allocation software with a randomized block procedure of size 4. Investigators, subjects and the statistician were blinded to treatment allocation. At the beginning of the study, a general questionnaire was completed for each participant. Patients in the intervention group (n=30) received 2 capsules per day containing 54 mg genistein for 12 weeks. The purity of genistein was >98%. The control group (n=30) received 2 placebo capsules per day for a similar duration. Placebo capsules contained 54 mg maltodextrin and were identical in size, color and appearance to the genistein capsules. All participants were recommended to maintain their usual dietary habits and physical activity throughout the study. A follow-up visit was performed once every 2 weeks, and compliance of the participants with the study protocol was monitored by recursive capsules. In addition, patients were checked for any possible complications during each follow-up visit.

Measurements

Body weight was measured using a seca scale (seca, Hamburg, Germany) to the nearest 0.1 kg, without shoes and wearing minimal clothing. Height was measured while the patient was barefoot, using a measuring tape to the nearest 0.1 cm. BMI was calculated as weight (kg)/height $(m)^2$. Waist circumference (WC) was measured midway between the lowest ribs and the superior border of the iliac crest, and hip circumference was recorded at the maximum circumference of the buttocks. Both measurements were taken with a flexible and nonstretchable tape to the nearest 0.5 cm. Waist-to-hip ratio (WHR) was calculated by dividing the WC by the hip circumference.

Dietary intake of subjects was assessed by 24-h recall method for 3 nonconsecutive days (2 weekdays and 1 weekend) at the beginning, middle and end of the study. Three-day average of energy and macronutrient intake were analyzed using Nutritionist 4 software (First Databank Inc, Hearst Corp, San Bruno, California, United States). The physical activity of each participant was obtained by a short and validated form of the International Physical Activity Questionnaire at baseline and the end of the study (25).

Blood sampling and laboratory assays

Venus blood samples from each participant were collected after 12-h overnight fasting for biochemical assessments before and after the intervention. Whole blood sample of 2 mL was conducted into a complete blood count tube containing ethylenediaminetetraacetic acid to assess A1C. The serum was separated by centrifugation and frozen at −70°C until further analysis. A1C was determined by ion exchange chromatography via a BioSystem A1C kit (BioSystems SA, Barcelona, Spain). Serum concentration of fasting glucose, total cholesterol (TC), triglyceride (TG) and high-density lipoprotein cholesterol (HDL-C) were measured by standard enzymatic method with a Pars Azmoon kit (Pars Azmoon, Karaj, Iran). Fasting insulin level was measured by an enzyme-linked immunosorbent assay method with the Monobind kits (Monobind Inc, Lake Forest, California, United States). Insulin resistance and insulin sensitivity were determined by HOMA-IR and quantitative insulin sensitivity check index (QUICKI) with the following respective formulas: HOMA-IR = fasting insulin (μU/L) × fasting blood glucose (mg/dL)/405, and QUICKI = 1/log insulin (μU/mL) + log glucose (mg/dL (26). Low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald formula as follows: LDL-C = TC (mg/dL) − ([HDL-C (mg/dL) − TG (mg/dL)]/5). Plasma TAC concentration was assayed with Randox kits (Randox Laboratories Ltd, Crumlin, County Antrim, United Kingdom). Serum level of MDA was measured using the thiobarbituric acid method. Biochemical measurements were also assessed at the end of the study.

Statistical analysis

Data analysis was performed using SPSS software version 16 (SPSS Inc, Chicago, Illinois, United States). The Kolmogorov-Smirnov test was performed to check normal distribution of variables. Analysis of variables with non-normal distribution, including HOMA-IR and insulin, was performed after logarithmic transformation. Independent sample *t* test and chi-square test were used to compare the baseline characteristics between the 2 groups. Paired sample *t* test was applied for within-group comparisons. The effect of the intervention was investigated by analysis of covariance adjusting for confounders. Repeated-measures analysis of covariance was performed to assess the within-group changes in dietary intake. The percent of change in each variable was determined as follows: [(after mean value − before mean value)/before mean value] × 100. The significance level was considered $p<0.05$.

Results

Sixty patients were enrolled in the study and 54 completed the 12-week intervention: 28 in the genistein group and 26 in the placebo group. The reasons for the loss to follow up are described in the study flow diagram (Figure 1). Compliance was good with >96% of the supplements being consumed in a prescribed manner during the study period. No adverse effects or symptoms were reported throughout the study.

Table 1 presents the baseline characteristics of the patients. There were no significant differences between the 2 groups in terms of age, time since menopause, duration of T2DM, using oral hypoglycemic agents and physical activity level at baseline. No significant change was observed in the use of medications and subjects' physical activity level throughout the study in any of the groups ($p>0.05$).

Daily dietary intake of subjects is shown in Table 2. No significant differences were seen in energy and macronutrient intake between the 2 groups at baseline. Changes in total energy and

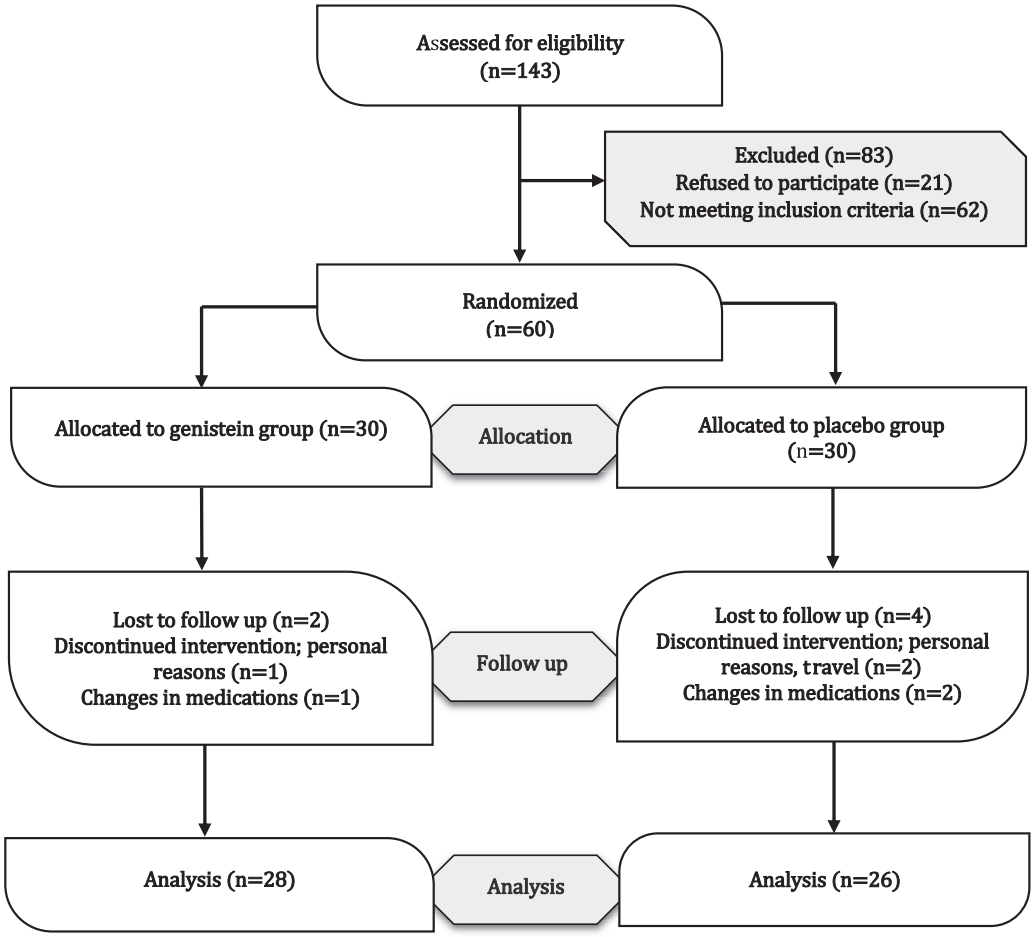

Figure 1. Study flow diagram.

macronutrient intake were not significant in either of the groups throughout the study ($p>0.05$).

Table 3 shows anthropometric characteristics of the participants at baseline and at the end of the intervention. There were no significant differences between the 2 groups in weight, BMI, WC, hip circumference and WHR at the beginning of the study. Within- and between-group changes in these variables were not significant in any of the groups at the end of the study.

Table 4 presents the biochemical parameters of subjects before and after the intervention. No significant differences were found for all biochemical measurements between the 2 groups at baseline ($p>0.05$). Significant decreases in serum levels of fasting glucose (by 17.02%, $p<0.001$), A1C (by 13.97%, $p<0.001$), TG (by 10.19%, $p=0.010$) and MDA (by 11.48%, $p=0.001$), and significant increases in QUICKI (by 4.32%, $p=0.042$), HDL-C (by 19.53%, $p=0.024$) and TAC (by 14.80%, $p<0.001$), were observed in the genistein group compared with baseline values. Supplementation with genistein resulted in an 11.16% reduction in HOMA-IR but was not significant. No significant differences were found in terms of all post-intervention values in the placebo group.

Table 1
Baseline characteristic of postmenopausal women with T2DM

Variable	Genistein group (n=28)	Placebo group (n=26)
Age, years	57.92±5.72	57.38±6.54
Duration of menopause, years	6.57±3.22	6.38±4.15
Duration of T2DM, years	3.64±1.56	3.38±2.28
Metformin 500 mg, tablets/day	2.21±0.62	2.19±0.56
Glibenclamide 5 mg, tablets/day	1.50±0.50	1.46±0.50
PAL		
Light	5 (17.9)	3 (11.5)
Moderate	23 (82.1)	23 (88.5)
Vigorous	0 (0.0)	0 (0.0)

PAL, physical activity level; *T2DM*, type 2 diabetes mellitus.
Note: Data are expressed as mean ± SD or n (%).

Table 2
Daily dietary intake of postmenopausal women with T2DM at baseline and 6 and 12 weeks after the intervention*

Variable	Genistein group (n=28)	Placebo group (n=26)
Total energy, kcal/d		
Before	1955.50±96.14	1949.92±96.25
Week 6	1956.85±92.21	1962.30±99.00
After	1973.64±80.14	1955.88±85.42
Carbohydrate, g		
Before	277.06±18.06	275.20±17.62
Week 6	276.37±15.96	275.00±19.63
After	276.52±15.43	275.45±16.39
Protein, g		
Before	79.13±10.28	80.40±6.76
Week 6	80.49±8.23	78.84±8.26
After	80.60±7.55	79.22±7.11
Fat, g		
Before	63.94±6.34	61.91±5.35
Week 6	63.10±5.45	64.68±6.11
After	64.52±5.20	63.90±4.53

T2DM, type 2 diabetes mellitus.
Note: Data are expressed as mean ± SD.
* Values are based on repeated-measures analysis of covariance ($p>0.05$).

Table 3
Anthropometric characteristics of postmenopausal women with T2DM at baseline and 12 weeks after the intervention

Variable	Genistein group (n=28)	Placebo group (n=26)	Mean differences (95% CI)	p value
Weight, kg				
Before	73.51±12.34	74.03±12.19	−0.52 (−7.22 to 6.18)	0.877*
After	73.23±12.65	74.06±12.77	−0.04 (−0.24 to 0.14)†	0.635‡
MD (95% CI)	−0.27 (−1.16 to 0.61)	0.02 (−0.51 to 0.56)		
p value	0.531§	0.919§		
BMI, kg/m^2				
Before	30.89±5.18	31.24±5.06	−0.35 (−3.15 to 2.44)	0.798*
After	30.77±5.26	31.25±5.27	−0.11 (−0.57 to 0.35)†	0.629‡
MD (95% CI)	−0.11 (−0.51 to 0.27)	0.01 (−0.22 to 0.23)		
p value	0.552§	0.994§		
WC, cm				
Before	96.77±11.22	97.20±9.25	−0.42 (−6.07 to 5.21)	0.897*
After	96.34±10.67	97.01±9.80	−0.17 (−2.07 to 1.72)†	0.855‡
MD (95% CI)	−0.43 (−1.93 to 1.07)	−0.19 (−1.47 to 1.08)		
p value	0.560§	0.760§		
HC, cm				
Before	108.01±10.16	108.07±9.42	−0.05 (−5.42 to 5.3)	0.982*
After	107.82±10.97	107.73±9.56	0.15 (−1.29 to 1.6)†	0.829‡
MD (95% CI)	−0.19 (−1.37 to 0.98)	−1.42 (−2.68 to −0.15)		
p value	0.735§	0.455§		
WHR				
Before	0.89±0.06	0.90±0.07	−0.005 (−0.04 to 0.03)	0.778*
After	0.89±0.06	0.90±0.07	−0.002 (−0.02 to 0.01)†	0.848‡
MD (95% CI)	−0.001 (−0.02 to 0.01)	0.009 (−0.001 to 0.02)		
p value	0.895§	0.940§		

BMI, body mass index; *CI,* confidence interval; *HC,* hip circumference; *T2DM*, type 2 diabetes mellitus; *WC,* waist circumference; *WHR,* waist-to-hip ratio.
Note: Data are expressed as mean ± SD or as otherwise indicated.
* p value based on independent sample *t* test.
† Analysis of covariance adjusted for baseline values and energy intake.
‡ p value based on analysis of covariance.
§ p value based on paired sample *t* test.

Based on analysis of covariance adjusted for baseline values, energy intake and BMI, significant differences were obtained in serum levels of fasting glucose (p=0.024), A1C (p=0.001), TG (p=0.025), MDA (p=0.016) and TAC (p=0.001) between the 2 groups at the end of the study.

Discussion

Genistein as abundant soy isoflavones has been investigated in some animal and human studies. Based on our literature review, this study is the first report about the possible effects of genistein in postmenopausal women with T2DM.

According to the results, dietary intake and physical activity levels of subjects did not change in both groups during the study. Therefore, these factors could not be accounted as confounding factors in the interpretation of other studied variables.

Our findings indicated that genistein supplementation for 12 weeks reduced serum fasting glucose and A1C levels compared with the placebo group, and improved insulin sensitivity in comparison with its baseline values. These findings are in agreement with the results of some animal and human studies (18,21,27–29). In a study on rats with streptozotocin-induced diabetes, oral administration of genistein (300 mg/kg/d) for 24 weeks decreased blood glucose and A1C (18). In another animal study, genistein supplementation in the diet of rats with streptozotocin-induced diabetes (600 mg/kg diet) for 3 weeks reduced blood glucose and A1C levels at 30 and 120 min after oral administration compared with the control group (27). Squadrito et al (21) reported that genistein supplementation with a dose of 54 mg/d for 1 year diminished fasting blood glucose and improved insulin resistance in postmenopausal women with metabolic syndrome. In a study by Villa et al (29), supplementation with 54 mg/d genistein for 24 weeks lowered fasting blood glucose, insulin and HOMA-IR in healthy postmenopausal women.

A number of studies have also reported the association between soy food consumption and reduction of the risk of T2DM (30–32). A cohort study by Villegas et al (31) demonstrated that soybean intake was inversely related to incidence of T2DM in Chinese women 40 to 70 years of age. In another cohort study by Nanri et al (32), an inverse association was observed between soy products intake and lower risk of T2DM in overweight Japanese women 45 to 75 years of age. Soybean products are the richest sources of isoflavones. Therefore, plasma isoflavones concentrations reflect the dietary intake of soybeans (33). Kwang-Pil et al (34) in a nested case-control study concluded a beneficial effect of a high intake of soybean products on risk of T2DM in women 40 to 69 years of age. As a result, high plasma concentration of genistein was associated with a decreased risk of T2DM in their study group (34).

It has been proposed that the effectiveness of genistein in glycemic control may be related to increasing glucokinase and decreasing glucose-6-phosphatase levels (27). In vitro and animal studies also have revealed that genistein binds to peroxisome proliferator-activated receptors (PPARs) (35,36). The gene-regulating PPARs play a pivotal role in insulin action and glucose metabolism.

It has also been demonstrated that estrogen regulates glucose disposal mainly via proteins of the insulin signalling pathways and consequently raises expression and translocation of glucose transporter 4 (GLUT4) (37). Similarly, Ha et al (38) determined that genistein increases glucose uptake by activation of adenosine monophosphate–activated protein kinase and induction of GLUT4 translocation. GLUT4 is the main glucose transporter in skeletal muscle cells and adipocytes and plays an important role in the maintenance of glucose homeostasis. Furthermore, genistein acts as a selective ER modulator and increases insulin sensitivity through binding to ERs (15). ERs are involved in modulating of glucose homeostasis and lipid metabolism (37).

Table 4
Biochemical characteristics of postmenopausal women with T2DM at baseline and 12 weeks after the intervention

Variable	Genistein group (n=28)	Placebo group (n=26)	Mean differences (95% CI)	p value
FBS, mg/dL				
Before	198.67±76.53	196.19±54.81	2.48 (−34.11 to 39.08)	0.892*
After	164.14±69.69	193.34±63.90	−31.81 (−59.34 to −4.28)†	0.024‡,§
MD (95% CI)	−34.53 (−46.82 to −22.24)	−2.84 (−30.59 to 24.9)		
p value	<0.001§,‖	0.834‖		
A1C, %				
Before	9.73±0.95	9.60±1.12	0.12 (−0.44 to 0.69)	0.662*
After	8.68±0.96	9.56±0.99	−0.93 (−1.43 to −0.42)†	0.001‡,§
MD (95% CI)	−1.04 (−1.46 to −0.62)	−0.04 (−0.48 to 0.39)		
p value	<0.001§,‖	0.845‖		
Insulin, μU/mL¶				
Before	9.71 (4.70 to 33.50)	9.64 (2.90 to 31.10)	−0.01 (−0.13 to 0.1)†	0.963*
After	9.09 (3.40 to 40.10)	9.57 (2.40 to 38.70)		0.805‡
p value	0.615‖	0.939‖		
HOMA-IR¶				
Before	4.45 (1.47 to 29.63)	4.50 (1.13 to 9.75)	−0.09 (−0.24 to 0.05)†	0.943*
After	3.39 (1.43 to 27.43)	4.33 (1.04 to 17.77)		0.220‡
p value	0.054‖	0.756‖		
QUICKI				
Before	0.30±0.02	0.30±0.02	0.0005 (−0.01 to 0.01)	0.940*
After	0.32±0.02	0.31±0.03	0.009 (−0.006 to 0.02)†	0.237‡
MD (95% CI)	0.01 (0.0005 to 0.02)	0.002 (−0.008 to 0.01)		
p value	0.042§,‖	0.631‖		
TC, mg/dL				
Before	186.78±49.64	190.26±35.56	−3.48 (−27.22 to 20.26)	0.770*
After	177.10±42.70	189.00±40.94	−10.85 (−30.95 to 9.24)†	0.283‡
MD (95% CI)	−9.67 (−28.15 to 8.79)	−1.26 (−14.95 to 12.41)		
p value	0.292‖	0.850‖		
TG, mg/dL				
Before	197.42±76.44	195.69±63.41	1.73 (−36.78 to 40.25)	0.928*
After	162.14±52.04	194.46±77.80	−34.4 (−64.26 to −4.55)†	0.025‡,§
MD (95% CI)	−35.28 (−61.35 to −9.21)	−1.23 (−23.09 to 20.63)		
p value	0.010§,‖	0.909‖		
HDL-C, mg/dL				
Before	32.73±11.31	33.78±8.79	−1.05 (−6.61 to 4.5)	0.705*
After	37.12±10.66	34.59±7.09	3.08 (−0.82 to 7)†	0.119‡
MD (95% CI)	4.39 (0.62 to 8.16)	0.8 (−1.53 to 3.14)		
p value	0.024§,‖	0.484‖		
LDL-C, mg/dL				
Before	140.75±41.37	144.37±37.22	−3.61 (−25.17 to 17.93)	0.737*
After	137.25±41.60	143.18±37.65	−4.24 (−22.43 to 13.94)†	0.64‡
MD (95% CI)	−3.5 (−19.3 to 12.3)	−1.18 (−13.11 to 10.74)		
p value	0.653‖	0.840‖		
TAC, mmol				
Before	1.37±0.21	1.44±0.33	−0.07 (−0.22 to 0.07)	0.333*
After	1.57±0.32	1.40±0.27	0.21 (0.07 to 0.34)†	0.003‡,§
MD (95% CI)	0.19 (0.1 to 0.29)	−0.03 (−0.14 to 0.06)		
p value	<0.001§,‖	0.449‖		
MDA, μmol/L				
Before	2.58±0.52	2.46±0.46	0.12 (−0.14 to 0.39)	0.366*
After	2.16±0.43	2.43±0.51	−0.32 (−0.66 to 0.02)†	0.016‡,§
MD (95% CI)	−0.42 (−0.65 to −0.19)	−0.02 (−0.25 to 0.2)		
p value	0.001§,‖	0.812‖		

A1C, glycated hemoglobin; *CI*, confidence interval; *FBS*, fasting blood glucose; *HDL-C*, high-density lipoprotein cholesterol; *HOMA-IR*, homeostatic model assessment of insulin resistance; *LDL-C*, low-density lipoprotein cholesterol; *MDA*, malondialdehyde; *QUICKI*, quantitative insulin sensitivity check index; *T2DM*, type 2 diabetes mellitus; *TAC*, total antioxidant capacity; *TC*, total cholesterol; *TG*, triglyceride.
Note: Data are expressed as mean ± SD or as otherwise indicated.
* p value based on independent sample *t* test.
† Analysis of covariance adjusted for baseline values, energy intake and body mass index.
‡ p value based on analysis of covariance.
‖ p value based on paired sample *t* test.
¶ Analyzed after log-transformation.
§ Statistically significant.

Our results confirmed the potential effects of genistein on lowering blood glucose and A1C. Considering the increase in QUICKI within the intervention group was significant, it is probable that a longer intervention period or higher dose of genistein might have been required to induce favourable changes in insulin sensitivity and insulin resistance in the study subjects.

Dyslipidemia is an important risk factor for atherosclerosis in patients with T2DM. In this study, genistein treatment decreased serum TG concentrations in comparison with the placebo group. Similar findings were reported in Kim et al (28) and Amanat et al (20) in their studies on genistein supplementation in mice with diet-induced nonalcoholic fatty liver disease (NAFLD) and on subjects with NAFLD, respectively. Squadrito et al (21) demonstrated a significant reduction in serum TC, LDL-C and TG levels, and increased HDL-C in postmenopausal women with metabolic syndrome on 54 mg genistein supplementation over 1 year.

Chronic elevated blood glucose promotes insulin resistance in adipose tissues and leads to enhancement of intracellular hydrolysis of TG and releasing of free fatty acids into circulation and the liver. Such condition persuades hypertriglyceridemia (39). Based on our findings, improved glycemia after genistein supplementation might be involved in lowering serum TG in studied patients. In addition, the effect of genistein on serum TG concentrations may be attributed to its effects on the enhancement of fatty acid catabolism in the hepatocytes (36). However, in the study by Choi et al (40), genistein supplementation in ovariectomized rats for 4 weeks did not alter serum lipid levels. No significant changes in serum TG and HDL-C were observed in osteopenic postmenopausal women after supplementation with 54 mg genistein aglycone for 3 years (41). Differences in study designs or metabolic characteristics of participants in clinical trials, and the dose and duration of the treatment period, might have contributed to controversial findings.

Diabetes is associated with an increase in reactive oxygen species (ROS) production, lipid peroxidation (42) and impaired antioxidant defenses (43). Lipid peroxidation generates a number of secondary products. MDA is the main and most studied toxic byproduct of polyunsaturated fatty acid peroxidation. Prior studies have shown that its plasma concentration is elevated considerably in T2DM (44). TAC is recognized as a reliable marker for measuring the cumulative action of all antioxidants in plasma and body fluids (45). Both TAC and MDA are markers for detecting oxidative stress status (46). According to our results, genistein supplementation remarkably decreased MDA levels and enhanced TAC levels compared with the placebo group. These findings are comparable with the results of some animal studies (18,23,24). An in vivo study conducted on low-density lipoprotein receptor knockout mice indicated that the genistein administration at 0.3 mg/kg body weight/d increased TAC and reduced MDA levels (24). A randomized controlled trial on patients with NAFLD showed that genistein supplementation with a dose of 250 mg/d significantly decreased MDA levels (20).

It has been demonstrated that genistein is capable of scavenging ROS and upregulating gene expression of antioxidant enzymes, such as superoxide dismutase, glutathione peroxidase, glutathione reductase and catalase (36,47). In addition, genistein reduces overproduction of proinflammatory cytokines via inhibition of the NF-κB pathway. Proinflammatory cytokines are important modulators of glucose homeostasis and lipid metabolism (48) and promote oxidative damage by the generation of ROS. In our study, there was a possibility that detected improvement in oxidative stress markers after genistein supplementation might be partly contributed to better glycaemia and TG levels in the intervention group, which was previously mentioned.

In this study, no significant differences were found in weight and anthropometric indexes between the 2 groups at the end of the trial. Similar results were reported in studies by Villa et al (29) and Squadrito et al (21) on healthy postmenopausal women or those with metabolic syndrome after genistein supplementation. However, decreased WC and WHR were observed in the study by Amanat et al (20) on NAFLD patients after genistein supplementation of 250 mg/d for 8 weeks. Kim et al (28) indicated that effect of genistein on body weight and fat mass may be attributed to the upregulation of fatty acid β-oxidation–related genes and the downregulation of genes associated with adipogenesis or lipogenesis, including sterol regulatory element-binding protein-1c, PPAR gamma, retinoid X receptor-a and acetyl CoA carboxylase 2. It was probable that dose or duration use of the supplement in our study subjects was not adequate to influence weight or obesity values significantly. On the other hand, it seems that detected marked biochemical results in our study patients were not mediated through changes in weight and other indexes of adiposity.

This study was designed as a randomized controlled trial that minimizes possible bias and confounding. However, this study had some limitations, including short study duration of 12 weeks and use of a fix dose of genistein. Therefore, the interpretation of the findings of this trial may not be applicable for using other doses of genistein or other intervention periods. Further studies are warranted to clarify the effects of genistein supplementation on obesity measurements in postmenopausal women with T2DM.

Conclusions

Genistein supplementation had favourable effects on glycemia, serum lipid profile and antioxidant status in postmenopausal women with T2DM and may be useful in management of diabetes complications. Genistein in dose and duration use in this study did not affect obesity values.

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Author Disclosures

Conflicts of interest: None.

Author Contributions

M.R. contributed to the study design and conceived the clinical trial progressing as a principal supervisor, prepared the manuscript and reviewed the whole project drastically. H.B. contributed to the data collection and data interpretation as a principal investigator and prepared the manuscript and reported the final results. S.K.H. contributed to the data collection and study conduction substantially. M.A.J. performed the statistical analysis and data interpretation. All authors read and approved the final version of the manuscript.

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