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Effects of Whey Protein Concentrate on Glycemic Status, Lipid Profile, and Blood Pressure in Overweight/Obese Women with Type 2 Diabetes Mellitus: A Randomized Placebo-Controlled Clinical Trial

ABSTRACT

Objective: Due to insufficient data on the metabolic consequences of long-term whey protein consumption, in this trial, we aimed to examine the effects of whey protein, as fortified bread, on glycemic status, lipid profile, and blood pressure in overweight/obese women with type 2 diabetes mellitus.

Methods: In a 12-week double-blind placebo-controlled randomized clinical trial, 48 overweight/obese women with type 2 diabetes mellitus were randomly allocated into either whey protein (bread fortified by 20 g whey protein concentrate) or placebo (unfortified bread) group. At pre- and post-intervention phase, physical activity, blood pressure, serum levels of glucose, insulin, glycosylated hemoglobin A1C, lipid profile, and dietary intakes were assessed. The homeostatic model assessment for insulin resistance was used for the estimation of insulin resistance.

Results: Totally 35 patients completed the trial. At the endpoint, there were no significant between-group differences for the assessed glycemic parameters ($P > .05$), except glycosylated hemoglobin A1C, which was higher in the whey protein group after adjusting for the confounders and baseline values ($P < .05$). Fasting blood glucose was significantly increased in whey protein group ($P < .05$). There was a significant increase in HOMA-IR and serum level of insulin in both whey protein and placebo groups ($P < .05$). There were no significant within- or between-group changes in the lipid profile and blood pressure of the patients ($P > .05$).

Conclusion: Three-month consumption of the whey protein concentrate fortified bread has no effects on lipid profile and blood pressure. It may cause some undesirable changes in some glycemic indices among overweight/obese women with type 2 diabetes mellitus.

Keywords: Blood pressure, diabetes mellitus, lipids, whey proteins

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Introduction

Diabetes mellitus (DM) is a group of diseases characterized by protracted high levels of blood glucose.[1] It is estimated that about 425 million adults around the world have DM and it is likely to about 629 million people aged 20-79 years develop DM in 2045.[2] Because of chronic complications of DM (microvascular and macrovascular complications), glycemic control is vital for patients with DM.[3-5]

Regarding the chronic nature of the disease, side effects of some hypoglycemic pharmacologic agents, and progressive tissue damage due to the poor control of diabetes, the researchers are motivated to seek remedies in alternative and traditional medicine that have milder toxicity than available synthetic drugs. Natural products from various sources, such as plants, functional foods, micronutrients, and various supplements, tend to be potential candidates for the prevention or treatment of DM and its related complications.[6]

Medical nutrition therapy is a part of diabetes care.[7] Some dietary factors such as whey protein (WP) have both insulinotropic and glucose-lowering effects in healthy subjects and patients with type 2 diabetes mellitus (T2DM).[8-10] Whey protein and casein comprise about 20% and 80% of the total protein in cow milk, respectively. β-lactoglobulin, α-lactalbumin, glycomacropeptide (GMP), lactoferrin, immunoglobulins, bovine serum albumin, and lactoperoxidase are components of WP.[11] Whey, which is produced in cheese-making process

as a by-product, is recognized as a functional food.[12] Whey protein concentrates (WPCs) and whey protein isolates (WPIs) with 35%-85% and >90% protein contents, respectively, and whey protein hydrolysate (WPH), which consists of proteins that are hydrolyzed by proteolytic enzymes, are different forms of WP.[11]

Some studies showed that WP can reduce postprandial blood glucose.[8,13] A systematic review of the acute (with less than 1 week) intervention studies on the effects of dairy foods and dairy proteins (casein and WP) in the management of T2DM concluded that despite beneficial effects of dairy foods and dairy proteins in T2DM care and glycemic control in acute interventions, long-term studies are needed.[14] In another review study, Stevenson et al[15] reported the improvement of glycemic control in obese, overweight, and normal weight subjects and patients with T2DM after acute WP supplementation, but they suggested further long-term intervention studies for considering WP supplementation as a therapeutic method.

Some studies showed that WP supplementation can reduce the serum levels of total cholesterol (TC), triglyceride (TG), and low-density lipoprotein cholesterol (LDL-C) in obese and overweight subjects and patients with T2DM.[16,17] Fekete et al[3] reported that WP lowers blood pressure (BP) and improves lipid biomarkers in adults with prehypertension and mild hypertension. Some other studies also showed beneficial effects of WP supplementation on high BP.[18,19]

Given the insufficient long-term clinical trials on the effects of WP on glycemic control, we conducted this 12-week randomized clinical trial (RCT) for examining long-term effects of the fortified bread by WPC on indices of glycemic control, lipid profile, and blood pressure among overweight/obese women with T2DM. Considering the probable desire of patients or healthy subjects to the consumption of natural compounds as a part of diet instead of powdered or capsulated supplements and with attention to the bread as a staple food of Iranians and also for increment of our intervention's applicability, we administered WP as whole wheat bread fortified with WPC.

Materials and Methods

Study Design and Subjects

The study was a double-blind, placebo-controlled RCT conducted for 12 weeks (90 days) between 2019 June and 2020 March. The patients were recruited from polyclinics, healthcare centers, and outpatient clinics of Tabriz University of Medical Sciences in Tabriz, Iran. Totally 48 overweight/obese women with T2DM aged 25-55 years and with a body mass index (BMI) of 25-40 kg/m^2 were initially enrolled.

The exclusion criteria were as follows: inflammatory, immunologic, pulmonary, and neoplastic diseases; uncontrolled thyroid, kidney, or liver disorders; malabsorption diseases such as ulcerative colitis or Crohn's disease; taking non-steroidal anti-inflammatory drugs, and glucocorticosteroid or hormonal drugs; use of insulin; any change in type or dose of administered drugs, and change in diet or physical activity (PA) during the intervention period; pregnancy, breastfeeding, and menopause; smoking; and allergy or intolerance to milk components. Written informed consent was obtained from each participant, and basic characteristics including demographic information and disease history were obtained from all patients. The primary outcomes of this study were changes in parameters of glycemic control. The secondary end-points were changes in lipid profile and BP.

This study was approved by the Ethics Committee of Tabriz University of Medical Sciences, Tabriz, Iran (ethics code: IR.TBZMED.REC.1397.687). This research was conducted according to the Declaration of Helsinki. The study was registered in the Iranian Registry of Clinical Trials (http://www.irct.ir, registration number: IRCT20110123005670N26).

Sample Size

For determining sample size, mean (standard deviation [SD]) of fasting blood sugar (FBS) was used from a previous clinical trial,[20] based on a confidence level of 95% and power of 90% in 2-sided tests. The sample size was calculated as 18 per group (WP and placebo groups) utilizing the Pockock formula, which was increased to 24, considering a probable of about 30% dropout rate.

Randomization and Intervention

A research assistant (the first author) randomly allocated the patients in a 1 : 1 ratio to either the WP or placebo group. The sequence of the randomization was generated utilizing the Random Allocation Software, considering randomized block procedure of size 2 [BMI ($\leq$ 32 kg/m^2 vs > 32 kg/m^2) and age ($\leq$40 years vs >40 years)]. The intervention allocation was blinded for participants and statisticians as well as investigators other than the first author. Based on the previous RCTs on WP supplementation[21-23] and considering a desirable formulation for whole wheat flat bread which was fortified with WPC for this research, 20 g WP (WPC 80 instant; Sachsenmilch Leppersdorf GmbH, 01454 Leppersdorf, Germany) was used for fortification of each bread. In addition, the achievement of a desirable formulation for the dough of whole wheat flour obliged us to use whole wheat flour (96% extraction rate) and white flour (82% extraction rate) in a ratio of 80 : 20, respectively. Patients in the WP group received 1 WPC fortified whole wheat flat bread (about 160 g) daily, while those in the placebo group received 1 whole wheat flat bread which was not fortified with WPC (about 125 g) for 12 consecutive weeks. The only difference between ingredients of breads was WPC; in each fortified brad, 20 g of flour was replaced with 20 g WPC. Notably, the difference in the weight of fortified and unfortified breads was due to WPC and higher amount of water, which were used for the preparation of the fortified bread. Table 1 shows the macronutrient composition of both kinds of breads in detail. All breads were prepared by a reference bakery (Athar Nan, Tabriz, Iran); all steps of the preparation and baking process were done under supervision of the investigator (the first author). Whey protein concentrate fortified and placebo breads were provided to both groups every 2 weeks.

An experienced dietician designed low-calorie diets for all patients according to the recommended dietary guidelines[1,7] and based on individualized characteristics of each participant. For designing these low-calorie diets, total energy expenditure was reduced depending on the individual characteristics and energy requirements of each patient. Macronutrient distribution in planned low-calorie diets was

MAIN POINTS

- 20 g/day whey protein did not have any significant effect on glycemic parameters.
- No significant effect was observed on blood pressure by 20 g/day whey protein.
- Lipid profile did not significantly improved by 20 g/day whey protein.
- For the improvement of metabolic parameters in T2DM, 20 g/day whey protein is not enough.

Table 1. Composition of WPC Fortified and Unfortified Breads

Sample of Bread	Energy (kcal/100 g)	Carbohydrate (g/100 g)	Protein (g/100 g)	Fat (g/100 g)	Fiber (g/100 g)
WPC fortified bread	223.7	37.72	14.02	1.86	5.37
Unfortified bread	251.91	50.14	8.9	1.75	6.14

WPC, whey protein concentrate.

not identical. For increasing PA, walking for at least 30 minutes a day was recommended for all of the patients. The patients were monitored every 2 weeks.

Anthropometric and Blood Pressure Measurements

One trained nutritionist performed the anthropometric measurements at baseline and after 12 weeks. The participants' height and weight were measured with a calibrated stadiometer and scale (Seca, Hamburg, Germany) to the nearest value of 0.1 cm and 0.1 kg, respectively. Body mass index was calculated as weight (kg) divided by height squared (m^2). One trained laboratory assistant measured blood pressure by an aneroid sphygmomanometer and stethoscope on the morning of the test day, at baseline, and endpoint. For a more accurate assessment, in pre- and post-intervention phase, we measured blood pressure of each participant twice with a 5-minute interval and reported the average of 2 values.

Assessment of Dietary Intake and Physical Activity

Dietary intake was estimated by 24-hour recall at baseline and end of the intervention period. Collected data on dietary intake were analyzed using the Nutritionist IV software (First Databank, San Bruno, Calif, USA) modified for Iranian foods. The PA of the patients was assessed by a validated international PA questionnaire-short form (IPAQ-SF).[24] Metabolic equivalent of task (MET)-minutes per week scores were calculated according to the guidelines for data processing and analysis of the IPAQ.[25] According to these guidelines, those subjects achieving a minimum total PA of at least 600 MET-minutes/week were considered to have a "moderate" PA level. The criterion for being classified as "high" PA level was achieving a minimum total PA of at least 3000 MET-minutes/week. Those patients who did not meet the 2 above-mentioned criteria were considered to have a "low" PA level.

Laboratory Assays

Following 12-hour overnight fasting, blood samples were collected in gel separator tubes (8 mL) and ethylenediamine tetraacetic acid (EDTA) blood collection tubes (2 mL). Blood sampling was performed at 7:30-9:00 AM in the Research Laboratory of the Faculty of Nutrition and Food Sciences, Tabriz University of Medical Sciences, Tabriz, Iran. The blood was sampled from "median cubital vein." For the separation of serum, blood samples collected in gel separator tubes were centrifuged at 2500 rpm for 10 minutes at 25°. The serum samples of each participant were stored in 6 0.5 mL micro-tubes at −80°. The enzymatic colorimetric method and commercial kits (Pars Azmoon Co., Tehran, Iran) were used for the measurement of FBS, TC, TG, and

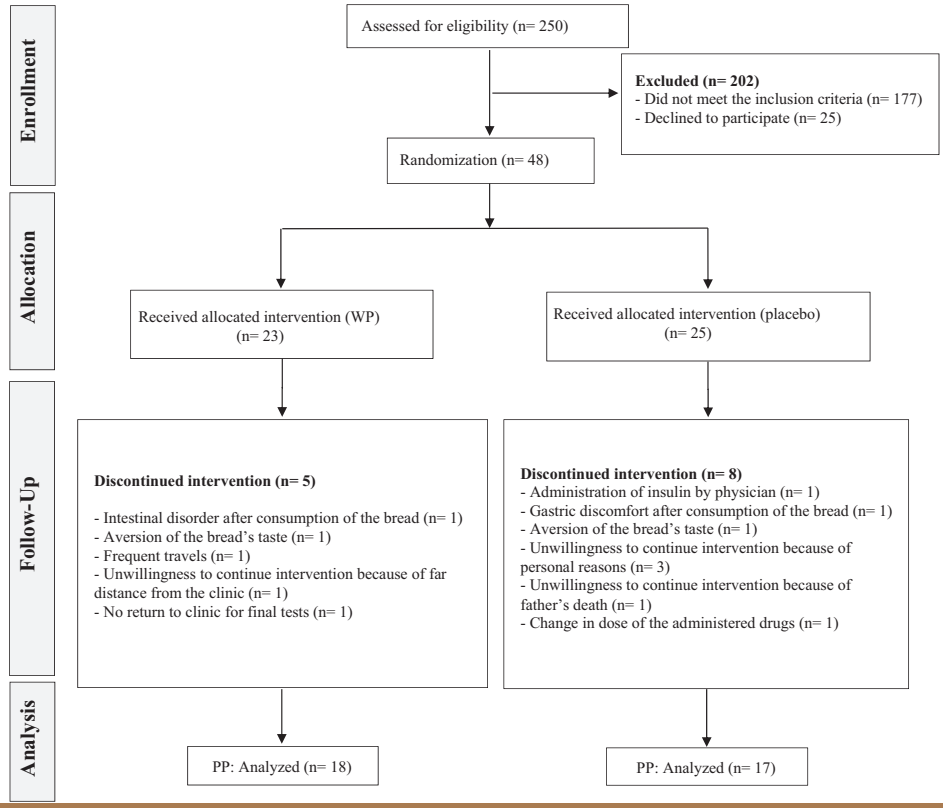

Figure 1. Study flow diagram. WP, whey protein; PP, per protocol.

high-density lipoprotein cholesterol (HDL-C) in serum. Serum LDL-C was calculated using Friedewald equation (LDL-C = TC – HDL-C – TG/5).[26] Enzyme-linked immunosorbent assay kit (100 N Pointe Dr, Lake Forest, CA 92630, Monobind Inc, USA) was used for the assessment of serum insulin concentration. Glycosylated hemoglobin A1C (HbA1C) was measured in whole blood samples collected in EDTA tubes, by auto-analyzer (Mindray Auto Hematology Analyzer) and using a commercial kit (BioRex Co., Tehran, Iran). The homeostatic model assessment for insulin resistance (HOMA-IR) was calculated via the following formula:

HOMA-IR = [fasting insulin (μIU/mL) × fasting glucose (mg/dL)]/405

Statistical Analyses

Statistical analyses were performed by International Business Machines Statistical Package for the Social Sciences Statistics software version 26 (IBM Corp.; Armonk, NY, USA). Analyses were conducted on a per protocol (PP) approach. To assess the normality of the data distribution, Kolmogorov–Smirnov test was run. Independent samples *t*-test was used for assessing between-group differences at baseline. For assessing within-group changes, paired samples *t*-test was applied. Fisher's exact test was used for the assessment of between-group differences in categorical variables. Analysis of covariance test was used for comparing the 2 groups at the end of study. We adjusted the analyses for baseline values and confounding factors (i.e., age, diabetes duration, administered drugs, BMI, PA, and intake of energy and macronutrients). $P < .05$ were considered statistically significant.

Table 2. Baseline Characteristics of the Study Participants

	WP (n = 18)	Placebo (n = 17)	*P*
Age (years)	44.00 (6.29)	46.94 (5.17)	.142[a]
Weight (kg)	81.88 (12.84)	80.01 (16.05)	.706[a]
Height (cm)	158.61 (7.70)	158.41 (6.44)	.935[a]
BMI (kg/m^2)	32.54 (4.26)	31.66 (5.04)	.579[a]
Education			
Illiterate	1 (5.55)	0 (0.0)	.562[b]
Diploma and lower	14 (77.77)	12 (70.58)	
Bachelors and higher	3 (16.66)	5 (29.41)	
Physical activity level			
Low (PA < 600 MET-minutes/week)	7 (38.88)	6 (35.29)	.896[b]
Moderate (PA > 600 MET-minutes/week)	7 (38.88)	7 (41.17)	
High (PA > 3000 MET-minutes/week)	3 (16.66)	4 (23.52)	
Drugs for glycemic control (metformin, diabezide)	18 (100)	17 (100)	
Drugs for dyslipidemia (atorvastatin)	9 (50)	10 (58.82)	
Drugs for hypertension (lozar)	7 (38.88)	7 (41.17)	

Age, weight, height, and BMI are presented as mean (SD); PA level and drugs use are presented as number (%). In the case of drug use, total number of participants using drugs are presented.
[a]Independent samples t-test.
[b]Fisher's exact test.
WP, whey protein; BMI, body mass index; PA, physical activity; SD, standard deviation; MET, Metabolic equivalent of task.

Results

General Characteristics of Trial and Dropouts

Totally 35 patients (18 in WP and 17 in placebo groups) completed the trial. The flowchart of the study is shown in Figure 1.

Demographic Characteristics

As shown in Table 2, at baseline, there were no significant differences between the 2 groups for age, weight, height, BMI, education level, marital status, and PA level. The mean age of the participants was 44.00 years in the WP group and 46.94 years in the placebo group.

Dietary Intakes and Physical Activities

As shown in Table 3, intake of energy, protein, carbohydrate, and daily percent of energy from fat were significantly decreased in placebo group ($P < .05$). A significantly lower protein intake was observed in the placebo group, when compared with the WP group after adjusting for the confounders. There was a significant increase in daily percent of energy from protein and a significant decrease in carbohydrate intake in WP group ($P < .05$). Within- or between-groups' changes of the PA (MET-minutes/week) were not significant over the study period (Table 3).

Biochemical Parameters and Blood Pressure

There was no significant difference between groups, for biochemical parameters and systolic blood pressure (SBP) as well as diastolic blood pressure (DBP) at baseline of the study (Table 4). As shown in Table 4, FBS was significantly increased in WP group ($P < .05$). A significantly higher HbA1C was observed in the WP group, when compared with the placebo group after adjusting for confounders and basal values. There was a significant increase in HOMA-IR and serum level of insulin in both WP and placebo groups. There were no significant within- or between-group changes for lipid profile, SBP, and DBP throughout the study (Table 4).

Discussion

Most of the previous clinical trials on WP supplementation examined the effects of short-term (less than 1 week) WP supplementation on glycemic control. We studied the long-term effects of WP in the more natural form of bread as main food items. In the present study, we found that daily intake of 20 g WP, as fortified bread, for 12 weeks had no beneficial effects on indices of glycemic control, lipid profile, and blood pressure in overweight/obese women with T2DM. Regarding indices of glycemic control, consumption of the fortified bread with WPC had no beneficial effect and just led to a significant increase in HbA1C after adjusting for the baseline values and confounders.

Our results are inconsistent with most of the previous short-term interventional studies. Recently, McDonald et al[27] assessed the effects of WP supplementation with 4 test beverages on the morning of 4 test days, in adults with prediabetes. They reported that the lowest glucose area under the curve (AUC) for 0-180 minutes was after consumption of WP beverage containing 16.5 g WP. In another study, Jakubowicz et al[21] examined the effects of WP drink (consisting of 50 g WPC and 250 mL water) for 2 test days in individuals with well-controlled T2DM; they showed lower glucose AUC (0-180 minutes) and higher insulin and C-peptide AUC (0-180 minutes) after WP consumption. Watson et al[23] assessed the effects of 4 different preloads in patients with T2DM and reported lower glucose AUC and higher insulin AUC after WP preload (containing 17 g WP) intake. Our results were in contrast with those short-term interventional trials which

Table 3. Daily Dietary Intakes and PA of the Study Participants Throughout the Study

Variable	Period	WP (n = 18)	Placebo (n = 17)	MD (95% CI), *P*
Energy (Kcal)	Baseline	1673.14 (679.45)	1808.47 (559.88)	−19.50 (−551.63, 512.62), .941[b]
	End	1469.12 (669.76)	1349.57 (463.66)	151.27 (−203.84, 506.39), .392[c], 0.164[d]
	MD (95% CI), *P*[a]	−204.02 (−587.31, 179.26), .276	−458.89 (−700.83, −216.95), .001	
Protein (g)	Baseline	60.73 (27.40)	68.47 (18.04)	7.74 (−8.31, 23.80), .334[b]
	End	62.14 (30.96)	51.46 (19.83)	13.13 (−4.67, 30.95), .143[c], .015[d]
	MD (95% CI), *P*[a]	1.41 (−15.91, 18.73), .865	−17.01 (−28.88, −5.13), .008	
Protein (percent of energy)	Baseline	13.55 (3.43)	15.23 (2.92)	1.67 (−0.52, 3.88), .130[b]
	End	16.33 (2.78)	15.00 (2.91)	1.39 (−0.66, 3.46), .178[c], .222[d]
	MD (95% CI), *P*[a]	2.77 (0.74, 4.81), .010	−0.23 (−2.44, 1.97), .824	
Carbohydrate (g)	Baseline	303.52 (142.66)	318.45 (101.84)	14.93 (−70.77, 100.63), .725[b]
	End	234.94 (97.94)	221.46 (74.54)	18.52 (−35.15, 72.20), .487[c], .584[d]
	MD (95% CI), *P*[a]	−68.58 (−134.44, −2.72), .042	−96.99 (−141.51, −52.47), <.001	
Carbohydrate (percent of energy)	Baseline	65.94 (9.57)	69.11 (7.49)	3.17 (−2.76, 9.11), .285[b]
	End	62.61 (8.00)	64.64 (6.66)	−1.59 (−6.78, 3.58), .535[c], .743[d]
	MD (95% CI), *P*[a]	−3.33 (−8.27, 1.61), .173	−4.47 (−10.07, 1.12), .110	
Fat (g)	Baseline	37.94 (22.58, 58.87)	29.06 (17.49, 40.54)	−0.08 (−0.28, 0.10), .368[b]
	End	31.98 (18.61, 68.19)	26.78 (18.72, 45.79)	−0.005 (−0.17, 0.16), .950[c], .376[d]
	MD, *P*[a]	−5.96, .439	−2.28, .945	
Fat (percent of energy)	Baseline	20.50 (9.06)	15.64 (6.14)	−4.85 (−10.21, 0.50), .74[b]
	End	21.05 (8.03)	20.35 (5.40)	−1.08 (−5.68, 3.50), .633[c], .417[d]
	MD (95% CI), *P*[a]	0.55 (−3.67, 4.78), .785	4.70 (0.94, 8.46), .017	
Fiber (g)	Baseline	14.56 (10.01, 22.10)	15.43 (10.94, 28.74)	0.05 (−0.12, 0.23), .539[b]
	End	18.62 (10.36, 22.08)	13.58 (7.83, 24.39)	0.009 (−0.17, 0.19), .925[c], .935[d]
	MD, *P*[a]	4.06, .837	−1.85, .355	
PA (MET-minutes/week)	Baseline	685.50 (267.50, 2571.00)	840.00 (259.00, 2939.75)	0.12 (−0.61, 0.86), .732[b]
	End	1071.00 (329.00, 2338.87)	1077.00 (675.00, 1968.00)	−0.06 (−0.38, 0.26), .709[c], .397[d]
	MD, *P*[a]	385.5, .374	237, .336	

Mean (SD) and mean difference (95% CI) are presented for normally distributed data; Median (25th and 75th percentiles) and median differences are presented for data not normally distributed (fat, fiber, and PA). Not normally distributed data are analyzed after log transformation.
[a]Paired samples *t*-test; [b]Independent samples *t*-test; [c]ANCOVA test, adjusted for baseline values (model 1); [d]ANCOVA test, adjusted for baseline values, age, diabetes duration, drugs, changes in BMI, intake of energy and macronutrients and PA (model 2).
WP, whey protein; PA, physical activity; METs, metabolic equivalent tasks (MET-minutes/week); SD, standard deviation; ANCOVA, analysis of covariance.

showed improvement in glycemic control after WP supplementation. Those short-term intervention studies administered WP supplements to 1 or more test days (acute administration) and reported postprandial amounts of glycemic parameters, while we measured serum levels of glycemic parameters in fasting state. So it seems that the discrepancy between the findings of our study and mentioned studies might result from the differences in the duration of intervention (acute or chronic administration) and measurement condition of glycemic parameters in serum (fasting or postprandial). In addition to the length of intervention, another reason for the result discrepancies between our study with other ones may be due to the differences in the type of fortified food.

There are a few long-term clinical trials that examined the effects of WP supplementation on glycemic control. In a 10-week intervention study, Gaffney et al[28] assessed the effects of a WPI beverage (containing 20 g WPI) in men with T2DM. Although they reported more reduction in FBS and HOMA-IR after consumption of WP beverage compared to the placebo, they showed likely and possible benefits on FBS and possible and unclear benefits on HOMA-IR in the WP and control groups, respectively.

It is believed that PA is a remarkable factor concerning glycemic control and insulin sensitivity.[29] Since the participants of Gaffney et al[28] study completed 45 high-intensity mixed-mode interval training (MMIT) sessions (27 cycling and 18 resistance training sessions) along with consumption of WP or placebo beverages, it seems that the high PA level of participants led to better outcomes for glycemic parameters in that study. In addition, the nutrients of foods, which were fortified with WP, could affect on metabolic characteristics of the WP.

In another long-term intervention study, Jakubowicz et al[20] examined the effects of 3 different types of breakfasts: whey breakfast diet (WBdiet), which contained 42 g protein of which 28 g were whey at breakfast; protein breakfast diet (PBdiet), which contained

Table 4. Biochemical Parameters and Blood Pressure of the Study Participants Throughout the Study

Variable	Period	WP (n = 18)	Placebo (n = 17)	MD (95% CI), *P*
FBS (mg/dL)	Baseline	154.50 (73.02)	127.82 (32.03)	−26.67 (−65.87, 12.51), .175[b]
	End	178.50 (67.78)	131.35 (55.26)	24.27 (−3.25, 51.80), .082[c], .452[d]
	MD (95% CI), *P*[a]	24.00 (4.37, 43.62), .019	3.52 (−16.38, 23.44), .712	
HbA1C (%)	Baseline	6.38 (1.46)	5.71 (1.00)	−0.67 (−1.54, 0.19), .123[b]
	End	7.26 (1.89)	5.98 (1.17)	0.87 (−0.14, 1.88), .090[c], .034[d]
	MD (95% CI), *P*[a]	0.87 (−0.06, 1.80), .066	0.27 (−0.18, 0.73), .224	
Insulin (μIU/mL)	Baseline	22.45 (5.75, 34.50)	16.30 (4.80, 31.80)	−0.05 (−0.35, 0.24), .697[b]
	End	44.30 (35.50, 50.95)	38.30 (31.50, 54.90)	0.006 (−0.13, 0.14), .924[c], .484[d]
	MD, *P*[a]	21.85, .004	22.00, .001	
HOMA-IR	Baseline	6.92 (2.35, 11.59)	5.31 (1.49, 9.54)	−0.11 (−0.40, 0.18), .454[b]
	End	20.45 (10.60, 28.00)	11.94 (8.64, 19.27)	0.14 (−0.03, 0.32), .116[c], .168[d]
	MD, *P*[a]	13.53, .001	6.63, .002	
TG (mg/dL)	Baseline	149.22 (57.97)	138.64 (53.94)	−10.57 (−49.14, 27.99), .581[b]
	End	179.33 (80.91)	157.88 (76.52)	10.83 (−27.95, 49.63), .573[c], .406[d]
	MD (95% CI), *P*[a]	30.11 (−2.92, 63.14), .071	19.23 (−1.29, 39.76), .064	
TC (mg/dL)	Baseline	151.05 (32.46)	145.64 (35.59)	−5.40 (−28.81, 17.99), .641[b]
	End	165.61 (48.36)	141.29 (36.65)	22.28 (−6.59, 51.17), .126[c], .130[d]
	MD (95% CI), *P*[a]	14.55 (−5.58, 34.69), 0.146	−4.35 (−30.98, 22.27), 0.733	
HDL-C (mg/dL)	Baseline	45.44 (8.61)	42.76 (10.48)	−2.67 (−9.26, 3.90), .413[b]
	End	50.50 (11.76)	45.35 (11.16)	4.05 (−3.57, 11.68), .287[c], .489[d]
	MD (95% CI), *P*[a]	5.05 (−0.83, 10.94), .088	2.58 (−3.85, 9.03), .407	
LDL-C (mg/dL)	Baseline	77.89 (25.05)	78.98 (29.36)	−0.63 (−19.58, 18.30), .946[b]
	End	85.24 (36.86)	67.01 (32.86)	12.22 (−12.29, 36.73), .318[c], .392[d]
	MD (95% CI), *P*[a]	3.47 (−11.74, 18.70), .636	−8.37 (−31.68, 14.93), .457	
SBP (mm Hg)	Baseline	120.00 (13.71)	116.61 (14.97)	−3.38 (−13.24, 6.48), .490[b]
	End	116.02 (19.91)	115.29 (12.08)	−0.714 (−11.57, 10.14), .894[c], .674[d]
	MD (95% CI), *P*[a]	−3.97 (−13.29, 5.35), .381	−1.32 (−9.53, 6.88), .737	
DBP (mm Hg)	Baseline	74.27 (8.58)	72.76 (3.19)	−1.51 (−6.01, 2.99), .499[b]
	End	72.86 (4.37)	71.73 (3.37)	1.11 (−1.64, 3.87), .417[c], .787[d]
	MD (95% CI), *P*[a]	−1.41 (−6.13, 3.30), .535	−1.02 (−3.50, 1.44), .391	

Mean (SD) and mean difference (95% CI) are presented for normally distributed data; Median (25th and 75th percentiles) and median differences are presented for data not normally distributed (insulin, HOMA-IR). Not normally distributed data are analyzed after log transformation.
[a]Paired samples *t*-test; [b]Independent samples *t*-test; [c]ANCOVA test, adjusted for baseline values (model 1); [d]ANCOVA test, adjusted for baseline values, age, diabetes duration, drugs, changes in BMI, intake of energy and macronutrients and PA (model 2).
WP, whey protein; FBS, fasting blood sugar; HbA1C, glycosylated hemoglobin A1C; HOMA-IR, homeostatic model assessment for insulin resistance; TG, triglyceride; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; SBP, systolic blood pressure; DBP, diastolic blood pressure; SD, standard deviation; ANCOVA, analysis of covariance.

42 g protein from various protein sources (eggs, tuna, and soy); and carbohydrate breakfast diet (CBdiet), which contained 17 g soy protein at breakfast, in adults with T2DM for 12 weeks. They found that the greatest reduction in FBS and HbA1C was achieved in WBdiet compared to the PBdiet and CBdiet. They also reported that the insulin AUC in WBdiet group was higher than PBdiet and CBdiet groups. It is shown that weight loss can improve glycemic control and T2DM.[30,31] The differences between the findings of our study and Jakubowicz et al's[20] study might be related to the higher dose of WP in WBdiet and a significant reduction in body weight which was observed in Jakubowicz et al[20] study. In addition, the HbA1C of our study participants was in normal range at baseline, so no changes in this parameter after consumption of WPC fortified bread are expected.

Our findings were in agreement with the results of a 2-week cross-over clinical trial among 22 patients with T2DM, which showed that WP supplementation (21 g WPI before breakfast and 21 g WPI before dinner) led to no significant differences in average glucose values.[32] Our results were also consistent with the results of a 12-week before-after study, which assessed the effects of WP supplementation in 31 overweight or obese patients with T2DM or impaired fasting glucose (IFG).[33] In that 12-week trial, administration of 20 g WPI before lunch and 20 g WPI before dinner resulted in no significant change in glucose markers (glucose, insulin, HbA1c, and HOMA-IR).

The current study showed that consumption of the fortified bread with WPC led to no significant within- or between-group changes in lipid profile. Pal et al[16] conducted a 12-week intervention on overweight/obese individuals and demonstrated that WP supplementation significantly lowered fasting lipids. The higher dose of WP and non-diabetic condition in the participants of Pal et al[16] study are probably related reasons for differences between the findings of our study and that study. On the other hand, the amounts of lipid

profile in participants of our study were in normal range at baseline, so it is expected that these parameters would not differ after consumption of the fortified bread by WPC. Our findings were also in contrast with the results of 2 review studies which showed that WP has beneficial effects on lipid profile.[34,35] It seems that high doses (45-75 g) of WP that was used in studies, which were assessed in those 2 reviews, might be the reason for this discrepancy. In a recent 3-month intervention study, Derosa et al[17] administered WPI for individuals with T2DM and found that TG, TC, and LDL-C decreased in the WPI group. The discrepancies between the results of our study and Derosa et al[17] study might have resulted from the difference in the dose of WP.

In the present study, we found that consumption of the fortified bread with WPC resulted in no significant within- or between-group changes for SBP and DBP of the participants. In an acute intervention study, Fekete et al[18] showed that WP supplementation reduced postprandial SBP compared with Ca-caseinate and maltodextrin up to 5 hours post-ingestion, in mildly hypertensive adults, but there was no significant change in postprandial DBP. Regarding DBP, our result was in line with the Fekete et al's[18] study, however, a contrast was observed, concerning SBP. This discrepancy between the findings might be related to the differences between the duration of our study (12-week intervention) and Fekete et al[18] study (acute intervention). In addition, the SBP and also DBP in the participants of our study were in normal range at baseline which may be the reason for no significant change in BP in our study, while the participants of Fekete et al[18] study were mildly hypertensive adults. Our findings were in line with the results of Yang et al[19] study on pre- and mildly hypertensive adults. They showed that consumption of 30 g/day WP for 12 weeks led to no significant change in SBP and DBP. Yang et al[19] also reported that SPB in WP group was significantly lower than control group, after dividing according to BMI. It seems that this finding might have resulted from the effect of body weight on blood pressure. Our results were also in agreement with the results of Flaim et al[33] study. They reported that supplementation with 40 g/day WPI for 12 weeks had no effect on SBP and DBP in patients affected by T2DM or IFG.

To the best of our knowledge, this study appears to be the first long-term RCT that used WP, as fortified whole wheat bread, for examining long-term effects of WP on indices of glycemic control, lipid profile, and blood pressure among overweight/obese women with T2DM. The main strength of our study was that dietary plans were provided based on the individual characteristics of each patient. In addition, for better monitoring and to increase the patients' motivation, we visited the participants every 2 weeks. Our trial had some limitations including subjective assessment of dietary intakes which usually does not represent the real intake accurately. Considering a desirable formulation for whole wheat bread fortified by 20 g WPC, we had to recommend a bread that weighted about 160 g, for daily consumption; so the patients should not intake other kinds of bread during the intervention period and appears that this could have influenced the patients' adherence after a while. For achieving significant effects on lipid profile, blood pressure, and also HbA1C, it might be more desirable that cut-offs were determined for these parameters at baseline.

Based on our findings, daily consumption of 20 g WPC, as fortified whole wheat bread, for 12 weeks had no significant beneficial effects on indices of glycemic control, lipid profile, and blood pressure in overweight/obese women with T2DM. Long-term consumption of fortified bread with WPC may cause some undesirable changes in some glycemic indices among overweight/obese women with T2DM. Further researches including a control group not receiving any interventions except individualized calorie-restricted diets are recommended to more obviously clarify the probable beneficial effects of WP fortified bread's intake on metabolic parameters in individuals with T2DM.

Data Sharing Statement: Individual deidentified participant data that underline the results reported in this article are available from the corresponding author upon reasonable request.

Ethics Committee Approval: This study was approved by the Ethics Committee of Tabriz University of Medical Sciences, Tabriz, Iran (ethics code; IR.TB ZMED.REC.1 397.6 87).

Informed Consent: Written informed consent was obtained from the patients who participated in this study.

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