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Article

Calorie Restriction Supplemented with Fish Oil Ameliorates Abnormal Metabolic Status in Middle-Aged Obese Women: An Open-Label, Parallel-Arm, Controlled Trial

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Abstract: The increasing prevalence of obesity and sedentary lifestyles has led to an increased incidence of metabolic syndrome (MetS) worldwide. In Taiwan, middle-aged women are at a greater risk of MetS, type 2 diabetes, and cardiovascular disease than men are because they have more subcutaneous fat and larger waist circumferences compared to men with equal visceral fat levels. This study investigated the effects of calorie restriction supplemented with fish oil (CRF) in middle-aged women with MetS. For 12 weeks, 75 eligible participants were randomly assigned either calorie restriction (CR) or CRF. Both dietary intervention groups were further divided into two age groups: ≤45 and >45 years. The changes in MetS severity, inflammatory status, iron status, and red blood cell fatty acid profile were evaluated. Seventy-one participants completed the trial. Both dietary interventions significantly ameliorated MetS and improved the participants' inflammatory status. CR significantly increased total iron binding capacity, whereas CRF increased hepcidin levels. Furthermore, CRF significantly increased the n-6/n-3 and arachidonic acid/docosahexaenoic acid ratios. In conclusion, CR and CRF improved the anthropometric and MetS characteristics of early-middle aged women, including body weight, blood glucose levels, triglyceride levels, as well as the scores for the homeostasis model assessment of insulin resistance and quantitative insulin sensitivity cheque index. Dietary intervention was more effective in >45-year-old women than ≤45-year-old women.

Keywords: calorie restriction; PUFA; meal replacement; metabolic syndrome; middle age

1. Introduction

Metabolic syndrome (MetS) has become a worldwide epidemic, particularly in the adult population, because of the increasing prevalence of obesity and sedentary lifestyles [1]. The characteristics of MetS are abdominal obesity, atherogenic dyslipidemia, elevated blood pressure (BP), insulin resistance (IR), and prothrombotic and pro-inflammatory status [2]. According to the National Health and Nutrition Examination Survey 2003–2012 data, the prevalence of MetS was 33% in the United States, with significantly higher prevalence in women than in men (35.6% vs. 30.3%; age ≥ 20 years). This prevalence was two times higher in middle-aged people (age, 40–50 years) than in 20–30-year-old people in the United States [3] and Taiwan [4]. Compared with middle-aged men, middle-aged women have a higher risk of MetS, type 2 diabetes (T2D), and cardiovascular disease (CVD) because of typically having more subcutaneous fat, which increases their waist circumference (WC) relative to subjects with equal visceral fat levels [5]. Thus, in Taiwan, the risk of abdominal obesity is greater in women than in men.

Several epidemiological studies have confirmed that MetS is highly associated with negative cardiovascular outcomes and all-cause mortality [6]. A recent systematic review and meta-analysis

found that MetS is associated with endometrial, pancreatic, breast, postmenopausal, rectal, and colorectal cancers in women [7]. Visceral obesity and IR have gained increasing attention as core manifestations of MetS. Visceral fat accumulation causes deregulation of adipocytokine production and secretion, particularly the reduction of adiponectin levels, which is the main mechanism for multiple risk development and a direct cause of CVD [5].

Extensive lifestyle interventions such as reduced energy intake and increased physical activity reduce MetS prevalence. A dietary approach for reducing MetS risk emphasises nutrient density, entailing limited consumption of whole grains and saturated and trans fats, increased consumption of a variety of fruit and vegetables, and the novel concept of discretionary calories [8]. Weight loss can be achieved by a conventional, structured, energy-restricted, modified diet—with or without meal replacement [9]. In addition, insulin sensitivity is affected by the quality and quantity of dietary fats, which independently affect body weight (BW). Studies have confirmed that a high intake of dietary saturated fat worsens IR. By contrast, n-3 polyunsaturated fatty acids (PUFAs) may prevent IR and delay MetS progression. Fish oils, containing n-3 PUFAs (particularly eicosapentaenoic acid [EPA] and docosahexaenoic acid [DHA]), are protective against several disease states. Dietary intake of fish oil may increase n-3 PUFA levels in the target tissues, thus rendering them more prone to peroxidative damage [10].

The increasing prevalence of MetS affects approximately one-fourth of the global adult population and is correlated with the global epidemic of obesity and T2D [11]. In some countries, the prevalence of MetS is higher in middle-aged women than in middle-aged men because women have more subcutaneous fat than men on average [5]. Reducing BW is positively correlated with reduction in MetS risk. Altering the macronutrient quality of a diet can aid in reducing BW and IR [9, 10, 12]. In this study, we focused on middle-aged women in Taiwan and investigated the effects of calorie restriction (CR) and CR with fish oil supplementation (CRF) on MetS characteristics. We also compared the degree of reduction in MetS severity in older and younger middle-aged women.

2. Materials and Methods

2.1. Participants

This study was conducted in Taiwan at the School of Nutrition and Health Sciences, Taipei Medical University (TMU) and its two affiliated hospitals, TMU Hospital and Wan Fang Hospital. Participants were recruited in Xinyi County, Taipei, Taiwan during May 2012–March 2013 through local community advertisements on the basis of our inclusion criteria.

In this 12-week trial, we included women aged 20 years or older, with body mass indices (BMIs) of 24–35 kg/m^2, WCs of ≥80 cm, and more than two of the following MetS signs, based on the modified National Cholesterol Education Program Adult Treatment Panel III [2] and World Health Organization [13] criteria: triglycerides (TGs) ≥ 150 mg/dL, high-density lipoprotein cholesterol (HDL-C) < 50 mg/dL, BP ≥ 130/ 85 mmHg, and fasting blood glucose (FBG) > 100 mg/dL.

The exclusion criteria were T2D, coronary heart disease, CVD, AIDS, smoking, alcoholism, pregnancy (or planning pregnancy), sensitivity or allergy to fish; use of lipid-lowering agents, antihypertension or hypoglycemic medications, and n-3 PUFA supplements; and neoplastic, renal, liver, endocrine, or psychiatric disease.

2.2. Study design

This was a single-centre, open-label, parallel-arm study to determine the effects of two dietary interventions in middle-aged women with MetS. The two dietary interventions were CR and CRF. Participants provided written informed consent and were then randomly assigned to two groups. At 0, 6, and 12 weeks after intervention, they underwent anthropometric, biochemical, clinical, and dietary assessments. In addition, for 1 h every week, they received a group teaching programme regarding healthy diet, determining fat content in food, skill required for eating out, behavioural changes, and exercise. Participants were asked to maintain daily dietary records; these were evaluated by a registered dietitian every week. This study was approved by the Joint Institutional

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Review Board of Taipei Medical University, conducted in accordance with the Declaration of Helsinki, and registered at ClinicalTrials.gov (NCT01768169).

2.3. Diet and dietary assessment

The registered dietitian measured the total daily energy requirements of the participants by determining their individual requirements at baseline. Basal metabolic rate (BMR) was calculated using the Harris–Benedict equation for women [14]: 655.1 + [9.6 × weight (kg)] + [1.9 × height (cm)] – [4.7 × age (years)]. BMR was then multiplied by 1.3 to estimate the total energy requirements for each participant. The macronutrient distribution of total calories was based on the 2011 Taiwanese dietary guidelines: 50%–55% from carbohydrates, 15%–20% from proteins, <30% from fat, and <300 mg/day cholesterol intake.

The CR group received CR only, whereas the CRF group received CR with Herbalifeline® n-3 PUFA-rich fish oil capsules (containing 2.13 g of n-3 PUFA) for partial lipid replacement. Each participant was administered 10 capsules/day, with each capsule containing 1,280 mg of EPA and 850 mg of DHA. Information regarding the nutritional composition of the diets is provided in Table 1.

Table 1. Nutritional composition of the CR diets (1,500 kcal)

Groups	CR	CRF
Calories (kcal)	1,483	1,528
Carbohydrates (g) (%)	192 (51.8%)	192 (50.3%)
Protein (g) (%)	64 (17.3%)	64 (16.8%)
Protein from meal replacement (g) (%)		
Fat (g) (%)		
Saturated fatty acids (g) (%)	21 (12.7%)	21 (12.4%)
PUFAs (g) (%)	30 (18.2%)	30 (17.6%)
n-3 PUFAs (g) (%)		5 (2.9%)

PUFA, polyunsaturated fatty acids; CR, calorie restriction; CRF, CR with fish oil supplementation.

The participants followed the guidelines of the Department of Dietetics at TMU Hospital during the 12-week intervention. Various calorie-controlled boxed lunches were provided to the participants. All participants were asked to prepare their own breakfasts. Sample menus and information on the calorie content of various breakfasts were provided to participants during the nutrition course meetings for instruction on healthy diets, exercise, dietary habits, and dietary behaviour modification. The course meetings were held every Friday afternoon by the registered dietitian. Each participant met the registered dietitian privately to address any additional dietary concerns. Participant compliance was measured by calculating the weight of the leftover CR and number of the leftover fish oil capsules, returned every 2 weeks.

2.4. Anthropometric measurements

The BMI (kg/m^2) of the participants was calculated using BW and body height during each of the two visits. BW was assessed to the nearest 0.1 kg by using a TANITA electronic scale (SC-330, Tokyo, Japan) and then height was measured to the nearest 0.5 cm by using a wall-mounted stadiometer, with the participants wearing light clothing and no shoes for both.

At every visit, anthropometric assessments and body fat percentage were calculated using a bioimpedance device (InBody 720, Biospace, Seoul, Korea). Body composition was estimated using a dual-energy X-ray absorptiometer (DEXA) scan obtained at the umbilical level in the supine position by an International Society for Clinical Densitometry-certified technologist. The DEXA scan data were analysed using Lunar enCORE 2006 software (version 10.50.086; GE Healthcare, Madison, WI, USA). The total body fat mass of the trunk, extremities, android and gynoid regions of each participant were determined using a Lunar Prodigy DEXA (GE Healthcare). All measurements were performed using the scanner at Wan Fang Hospital; the concerned hardware and software were not changed during the trial.

2.5. Analysis of clinical and biochemical variables

Clinical and biochemical data were collected on each visit of the participants. BP was measured three times from the right arm of a participant in the sitting position after a 5-min rest by using an Omron HEM-7230 automatic sphygmomanometer (Kyoto, Japan). The measurements were averaged to determine mean arterial pressure (MAP), systolic BP (SBP), and diastolic BP (DBP) [15].

Glycaemic responses were assessed through 2-h oral glucose tolerance tests, blood glucose levels, and serum insulin levels. Blood samples were taken at 0 (baseline), 6, and 12 weeks after intervention through a catheter in an antecubital vein after a 12-h overnight fast. Blood glucose and serum insulin levels were specifically analysed at 0, 15, 30, 45, 60, 90, and 120 min after participants consumed 75 g of glucose. Blood samples were stored at −80°C before analysis. For assessing IR, we used the homeostasis model assessment of IR (HOMA-IR), applying a glucose clamp technique and the quantitative insulin sensitivity cheque index (QUICKI). The formula for HOMA-IR is fasting plasma insulin (μIU/mol) × fasting plasma glucose (mol/L)/22.5. The QUICKI is derived from FBG and insulin levels: 1/[log fasting insulin (μU/mL) + log FBG (mg/dL)] [16]. Blood glucose levels were examined with an automated analyzer (VITOR 5, 1FS, Ortho Clinical Diagnostics, Johnson and Johnson) and Victors Chemistry Products GLU slides using a hexokinase method. Serum insulin was determined using a radioimmunoassay kit (DIA Source, Lovain-La-Nueve, Belgium). Haemoglobin A1c (HbA1c) levels were analysed using an HLC-723 GHb G7 analyser (Tosoh, Tokyo, Japan).

The total protein, albumin, HbA1c, C-reactive protein (CRP), interleukin-6 (IL-6), TG, total cholesterol (TC), low-density lipoprotein-cholesterol (LDL-C), and HDL-C levels, as well as the liver function index (glutamic oxaloacetic transaminase [GOT] and glutamic pyruvic transaminase [GPT]) and kidney function index (blood urea nitrogen and creatinine) were measured from the serum samples. The serum albumin, total protein, TG, and TC levels were determined using an Ortho Clinical Diagnostics VITROS 950 (Johnson and Johnson, New Brunswick, NJ, USA). The serum LDL-C and HDL-C levels were evaluated using a Toshiba TBA-c 16000 (Toshiba, Tokyo, Japan). The IL-6 levels were examined using a Human IL-6 Quantikine ELISA Kit (R&D Systems, Minneapolis, MN, USA). The serum CRP levels were determined using a Toshiba TBA-c 16000 (Toshiba). Serum ferritin levels were measured using a commercially available electrochemiluminescence immunoassay and quantitated on a Roche Modular P800. Serum iron and total iron binding capacity (TIBC) were estimated photometrically by using a ferrozine-based iron and unsaturated iron binding capacity reagent (Randox Diagnostics, UK). The transferrin saturation percentage was calculated as serum iron/TIBC × 100%. Serum hepcidin levels were assessed using an enzyme-linked immunosorbent assay (DRG International Inc, Marburg, Germany). The MetS Z-score is an indicator of MetS severity, assigned using the five MetS characteristics. For women, the Z-score is calculated as [(50 – HDL)/12.4] + [(TGs – 150)/66.5] + [(FBG – 100)/13.4] + [(WC – 88)/11.7] + [(MAP – 100)/10.03] [16].

2.6. Analysis of red blood cell fatty acid profile

Red blood cell (RBC) fatty acid profiles were extracted using a previously described modified method [17]. Blood samples were collected and centrifuged to obtain plasma samples, which were stored at −80°C until biochemical analysis. The fatty acid methyl ester profiles of the plasma samples were analysed through gas chromatography (GC), following methods described previously [18]. Washed RBCs (500 mg) and frontal cortices (20 mg) were mixed with distilled water–methanol–chloroform (2:2:3, v/v/v) and saponified with a saturated saline solution (2 mL). The phospholipids were separated using solid phase extraction columns (J.T. Baker, Center Valley, PA, USA). Fatty acid methylation was performed by heating at 100°C for 10 min with a boron trifluoride-methanol reagent (15%, 0.3 mL). The extracted lipids were precipitated to powder through vacuum filtration. Fatty acid methyl esters were analysed using a capillary gas chromatograph (Trace GC, Thermo Finnigan Trace GC, Milan, Italy) equipped with a capillary column of 30 m length, 0.32 mm inner diameter, and 0.32 mm df (Rtx®-2330 column, Restek, Bellefonte, PA, USA) and a frame ionisation detector. The GC oven temperature was initially maintained at 160°C, then increased at 5°C/min to 250°C, after which it was maintained at 250°C for 5 min; the injector and detector were both maintained at 260°C. Fatty acid

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profiles were identified according to the retention time of the appropriate standard fatty acid methyl esters (Supelco, St. Louis, MO, USA).

The composition data were calculated as the peak percentages of the total fatty acids. The n-6 and n-3 PUFAs and the n-6/n-3 ratio were determined as previously described [19]. The examined n-6 PUFAs included linoleic acid (LA, C18:2), eicosadienoic acid (C20:2), and arachidonic acid (AA, C20:4); the n-3 PUFAs examined included α-linolenic acid (ALA, C18:3), EPA (C20:5), docosapentaenoic acid (DPA, C22:5), and DHA (C22:6).

2.7. Statistical analysis

Data are presented as the mean ± standard error of the mean of each variable. Differences between the baseline characteristics of the treatment groups were compared using the Student *t* test. The differences between the two groups were examined using one-way analysis of variance. Changes from the baseline to the study endpoint in the RBC, ALA, EDA, EPA, DPA, and DHA levels and changes in the RBC ratios of n-6/n-3, AA/EPA, and AA/DHA were compared using a one-way analysis of variance. A Pearson correlation coefficient was calculated to evaluate the relationship between the cardiometabolic parameters and MetS severity Z-scores. All statistical analyses were performed using SAS software (version 9.1.4; SAS Institute, Cary, NC, USA).

3. Results

3.1. Participant characteristics

Figure 1 shows a flowchart of participant selection. Initially, 278 women applied to participate in this study; however, 203 participants were excluded because they did not fulfil the MetS criteria. A total of 75 eligible participants were randomly assigned to either the CR or CRF groups for 12 weeks. Forty participants were assigned to CR, whereas 35 were assigned to CRF. Four participants withdrew for various reasons: relocation (n = 1), unspecified personal reasons (n = 2), and family-related reasons (n = 1). The remaining 71 participants completed the 12-week trial. The two groups demonstrated nonsignificant differences in age (52.0 ± 11.5 years) and baseline BMI (29.5 ± 4.0 kg/m^2). In addition, the participants exhibited no significant changes in nutritional status, hepatic profiles, renal function, or routine haematological parameters throughout the course of the trial. The dietary interventions were well tolerated by all participants and no adverse events occurred during the trial.

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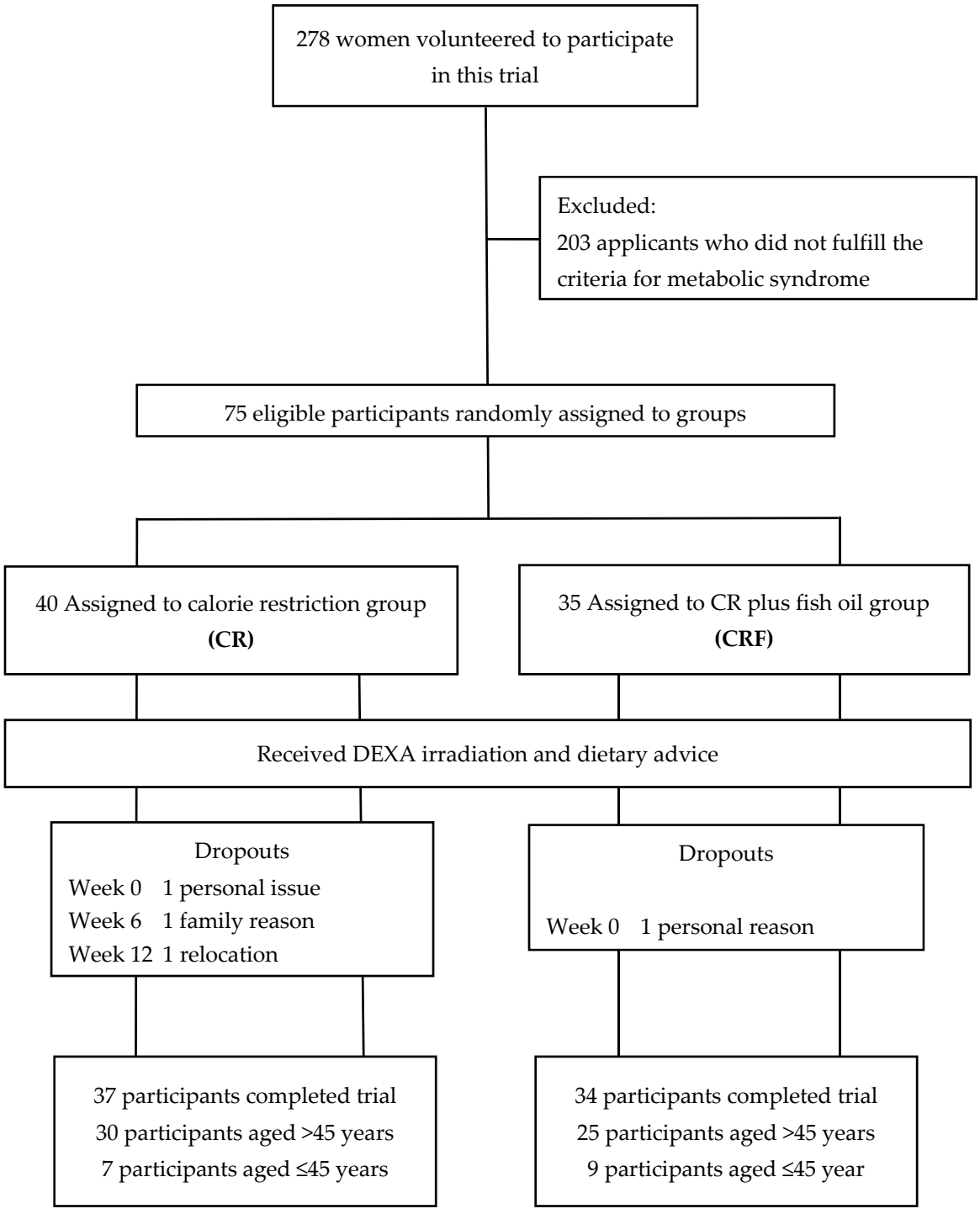

Figure 1. Flowchart of participant selection. Participants were assigned to one of the following dietary groups: calorie restriction (CR) and CR with fish oil supplementation (CRF). The 12-week trial was completed by 71 participants.

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3.2. Changes in anthropometric characteristics

The 12-week CR and CRF interventions affected the patients' anthropometric profiles, body composition, liver function, blood sugar, blood lipid, inflammatory status, serum iron status, severity of MetS (Z-score), and RBC fatty acids profiles. For the two groups, both CR and CRF for 12 weeks significantly reduced the BW (−2.3 to −3.7 kg), WC (−6.4 to −9.5 cm), SBP, DBP, and MAP from prehypertension to normal levels ($p < 0.001$). In addition, the BMI of the participants in both groups decreased significantly ($p < 0.001$). CRF more effectively reduced WC than CR did (up to 9.5 cm; $p < 0.001$).

Table 2. Anthropometric blood pressure and body composition characteristics before and after dietary interventions

Variables	CR (n = 37)			CRF (n = 34)			
	Baseline	Week 12	*p*	Baseline	Week 12	*p*	Posttreatment *p*
Anthropometrics							
BW (kg)	74.6 ± 2.0	72.3 ± 2.1	<0.001*	73.9 ± 2.0	70.2 ± 1.9	<0.001*	<0.001**
BMI (kg/m²)	29.8 ± 0.8	28.9 ± 0.8	<0.001*	29.4 ± 0.5	27.9 ± 0.5	<0.001*	<0.001**
WC (cm)	92.7 ± 1.5	86.3 ± 1.5	<0.001*	92.6 ± 1.4	83.1 ± 1.4	<0.001*	<0.001**
Blood pressure							
SBP (mmHg)	141.5 ± 2.7	129.9 ± 2.7	<0.001*	140.7 ± 2.6	125.3 ± 2.5	<0.001*	<0.001**
DBP (mmHg)	86.4 ± 1.7	79.5 ± 1.6	0.001*	85.3 ± 1.8	78.8 ± 1.8	0.001*	<0.001**
MAP (mmHg)	104.8 ± 1.8	96.3 ± 1.7	<0.001*	103.8 ± 1.9	94.3 ± 2.0	<0.001*	<0.001**
Body composition							
Android fat (%)	50.2 ± 0.7	49.2 ± 0.8	0.021*	50.7 ± 0.9	48.9 ± 0.9	<0.001*	<0.001**
Gynoid fat (%)	45.3 ± 0.7	44.6 ± 0.8	0.040*	46.4 ± 0.9	45.2 ± 1.0	0.006*	<0.001**
Android/Gynoid	1.11 ± 0.02	1.11 ± 0.02	0.495	1.10 ± 0.02	1.11 ± 0.03	0.667	<0.001**
Total body fat (%)	42.0 ± 0.8	41.3 ± 0.9	0.031*	42.9 ± 1.0	41.4 ± 0.9	<0.001*	<0.001**
Lean/Fat ratio	1.41 ± 0.05	1.46 ± 0.05	0.169	1.37 ± 0.05	1.42 ± 0.07	<0.001*	<0.001**
Laboratory data							
Total protein (g/dL)	7.55 ± 0.07	7.04 ± 0.07	<0.001*	6.99 ± 0.06	7.34 ± 0.07	<0.001*	<0.001**
Albumin (g/dL)	4.26 ± 0.03	4.28 ± 0.03	0.426	4.29 ± 0.03	4.18 ± 0.03	0.001*	<0.001**
GOT (μ/L)	25.6 ± 1.6	27.2 ± 2.3	0.504	29.4 ± 2.5	28.8 ± 3.2	0.747	<0.001**
GPT (μ/L)	30.0 ± 3.4	26.4 ± 2.4	0.142	36.2 ± 6.2	33.8 ± 7.9	0.540	<0.001**
BUN (mg/dL)	14.8 ± 0.6	14.4 ± 0.7	0.430	13.9 ± 0.7	12.5 ± 0.4	0.014*	<0.001**

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Creatinine (mg/dL)	0.67 ± 0.02	0.69 ± 0.02	0.196	0.67 ± 0.02	0.71 ± 0.02	0.001*	<0.001**
Uric acid (mg/dL)	6.00 ± 0.21	5.94 ± 0.23	0.656	5.62 ± 0.20	5.81 ± 0.17	0.283	<0.001**

BMI, body mass index; BW, body weight; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure. Android/Gynoid, Android fat and Gynoid fat ratio; Lean/Fat ratio, lean body mass and fat body mass ratio; GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvic transaminase; BUN, blood urea nitrogen. Data are presented as mean ± standard errors of the mean. *Significantly different from baseline by paired t test, $p < 0.05$. **Significantly different by t test, $p < 0.05$.

3.3. Changes in clinical and biochemical variables

For body composition profiles, both 12-week interventions reduced the android, gynoid, and total body fat percentages to approximately 0.8%–1.5% and increased the lean mass/fat mass ratio ($p < 0.05$). Assessment of the nutritional status, liver function, and renal function showed that the levels of the selected parameters (total protein, albumin, GOT, GPT, BUN, and creatinine) were within normal biochemical ranges. The dietary interventions were well tolerated by all participants, and no adverse events occurred during the trial (Table 2).

Decreased blood glycaemic responses including the FBS, PC, insulin, and HbA1c levels, as well as the HOMA-IR and QUICKI were observed after the 12-week interventions (all $p < 0.05$). Moreover, significant reduction of the TG, LDL-C, CRP, IL-6, and Z-score to normal levels, as well as significantly increased HDL-C levels, were observed after 12 weeks (all $p < 0.05$; Table 3).

Both interventions reduced the MetS severity, based on the Z-scores; the severity was significantly associated with inflammatory status. In the MetS patients, only CR reduced the TIBC ($p < 0.05$), whereas only CRF reduced the hepcidin levels ($p < 0.05$). Furthermore, after 12 weeks, CRF significantly increased the levels of DHA and n-3 PUFA, as well as reducing the n-6/n-3 and AA/DHA ratios (all $p < 0.05$; Table 4).

Table 3. Blood sugar, lipid profile, inflammatory status, iron status, and Z-score at baseline and 12 weeks after the dietary interventions

Variable	CR (n = 37)			CRF (n = 34)			
	Baseline	Week 12	*p*	Baseline	Week 12	*p*	Posttreatment *p*
Blood sugar							
FBS (mg/dL)	109.4 ± 3.7	101.4 ± 2.7	0.005*	104.4 ± 1.6	94.4 ± 1.1	<0.001*	<0.001**
PC (mg/dL)	147.4 ± 9.6	123.2 ± 7.7	0.001*	142.7 ± 7.7	98.2 ± 4.3	<0.001*	<0.001**
Insulin (μIU/mL)	17.2 ± 1.3	11.5 ± 1.0	<0.001*	14.4 ± 1.0	11.4 ± 0.8	0.009*	<0.001**
Hb (g/ dL)	13.6 ± 0.2	13.9 ± 0.2	0.012*	13.7 ± 0.2	13.9 ± 0.2	0.358	<0.001**
Hb_{A1c} (%)	6.06 ± 0.09	6.08 ± 0.09	0.957	6.05 ± 0.07	5.97 ± 0.06	0.037*	<0.001**
HOMA-IR	4.65 ± 0.37	2.89 ± 0.26	<0.001*	3.87 ± 0.26	2.67 ± 0.19	<0.001*	<0.001**
QUICKI	0.31 ± 0.00	0.33 ± 0.00	<0.001*	0.32 ± 0.00	0.33 ± 0.00	0.001*	<0.001**
Blood lipid							
TG (mg/dL)	149.6 ± 14.9	145.4 ± 16.7	0.600	152.3 ± 11.1	106.6 ± 4.8	<0.001*	<0.001**

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TC (mg/dL)	197.4 ± 4.9	197.9 ± 5.7	0.861	200.4 ± 6.8	214.6 ± 6.5	0.003*	<0.001**
HDL-C (mg/dL)	48.2 ± 1.2	49.6 ± 1.4	0.064	50.1 ± 2.0	52.7 ± 2.0	0.029*	<0.001**
LDL-C (mg/dL)	122.6 ± 4.1	116.9 ± 5.1	0.041*	131.8 ± 6.9	130.4 ± 5.6	0.726	<0.001**
Inflammatory status							
CRP (mg/dL)	0.45 ± 0.11	0.37 ± 0.06	0.464	0.48 ± 0.08	0.33 ± 0.06	<0.001*	<0.001**
IL-6 (pg/mL)	3.36 ± 0.37	2.83 ± 0.45	0.106	3.83 ± 0.54	1.61 ± 0.21	<0.001*	<0.001**
Iron serum							
Fe (µg/L)	73.4 ± 4.3	78.6 ± 3.8	0.335	68.3 ± 5.4	76.9 ± 5.2	0.137	<0.001**
TIBC (µg/L)	285 ± 7.4	303 ± 7.8	0.002*	305 ± 8.9	309 ± 7.4	0.579	<0.001**
Ferritin (ng/L)	111.0 ± 13.7	116.3 ± 14.8	0.743	110.3 ± 24.9	109.4 ± 25.8	0.910	<0.001**
Hepcidin (ng/L)	732.6 ± 4.1	734.8 ± 3.6	0.480	715.6 ± 5.1	723.6 ± 4.2	0.015*	<0.001**
Z-score	1.66 ± 0.51	−0.46 ± 0.51	<0.001*	0.98 ± 0.35	−2.19 ± 0.36	<0.001*	<0.001**

FBS, fasting blood sugar; PC, postprandial glucose test (2 h after eating); Hb, haemoglobin; HbA1c, haemoglobin A1C; HOMA-IR, homeostasis model of assessment for insulin resistance index; QUICKI, the quantitative insulin sensitivity cheque index; TG, triglyceride; TC, total cholesterol; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein; CRP, C-reactive protein, IL-6, interleukin-6; Fe, iron; TIBC, total iron-binding capacity. Data are presented as means ± standard errors of mean. *Significantly different from baseline by paired t test, $p < 0.05$. **Significantly different by t test, $p < 0.05$.

Table 4. RBC fatty acids profile at baseline and 12 weeks after the dietary interventions

Variables	CR (n = 30)			CRF (n = 29)			Posttreatment
	Baseline	Week 12	*p*	Baseline	Week 12	*p*	*p*
RBC fatty acids (%)							
ALA (n-3)	11.5 ± 0.6	12.6 ± 0.6	0.121	11.5 ± 0.7	13.5 ± 0.7	0.074	<0.001**
AA (n-6)	3.12 ± 0.21	4.86 ± 0.97	0.109	8.09 ± 1.33	6.24 ± 0.96	0.285	<0.001**
EPA (n-3)	1.02 ± 0.15	0.86 ± 0.14	0.351	1.61 ± 0.27	2.11 ± 0.34	0.213	<0.001**
DPA (n-3)	0.42 ± 0.05	0.86 ± 0.14	<0.001*	1.49 ± 0.42	2.11 ± 0.34	0.240	<0.001**
DHA (n-3)	1.78 ± 0.26	2.58 ± 0.36	0.100	1.39 ± 0.18	3.21 ± 0.24	0.029*	<0.001**
n-3 PUFAs	12.3 ± 0.8	14.5 ± 0.7	0.052	11.9 ± 0.8	16.8 ± 0.8	<0.001*	<0.001**
n-6 PUFAs	15.5 ± 0.6	19.9 ± 1.3	0.008*	21.0 ± 1.4	20.7 ± 1.0	0.824	<0.001**

n-6/n-3	1.41 ± 0.10	1.68 ± 0.28	0.350	1.96 ± 0.18	1.36 ± 0.11	0.005*	<0.001**
AA/EPA	4.76 ± 0.63	8.96 ± 2.11	0.080	9.95 ± 2.64	14.9 ± 6.9	0.468	0.001**
AA/DHA	3.93 ± 1.27	14.9 ± 8.36	0.219	9.59 ± 1.62	2.94 ± 0.61	0.005*	0.047*

AA, arachidonic acid; ALA, alpha-linolenic acid; LA, linoleic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; PUFA, polyunsaturated fatty acid. Data are presented as means ± standard errors of mean. *Significantly different from baseline by paired t test, $p < 0.05$. **Significantly different by t test, $p < 0.05$.

3.4. Differences in the characteristics between younger and older middle-aged women

BW decreased significantly in younger and older middle-aged women in both groups. Both interventions significantly improved the body composition, including android, gynoid, and total body fat percentages and lean mass/fat mass ratios (all $p < 0.05$).

The changes in anthropometric and laboratory characteristics after the 12-week dietary interventions significantly differed between younger and older middle-aged women with respect to their BMIs; android, gynoid, and total body fat percentages; total protein, albumin, PC, HbA1c, TG, TC, LDL-C, and IL-6 levels; Z-scores; and TIBCs (all $p < 0.05$; Table 2 and Figure 2). Regarding inflammatory statuses, compared with CR, CRF more effectively reduced CRP and IL-6 levels in older participants than in younger participants.

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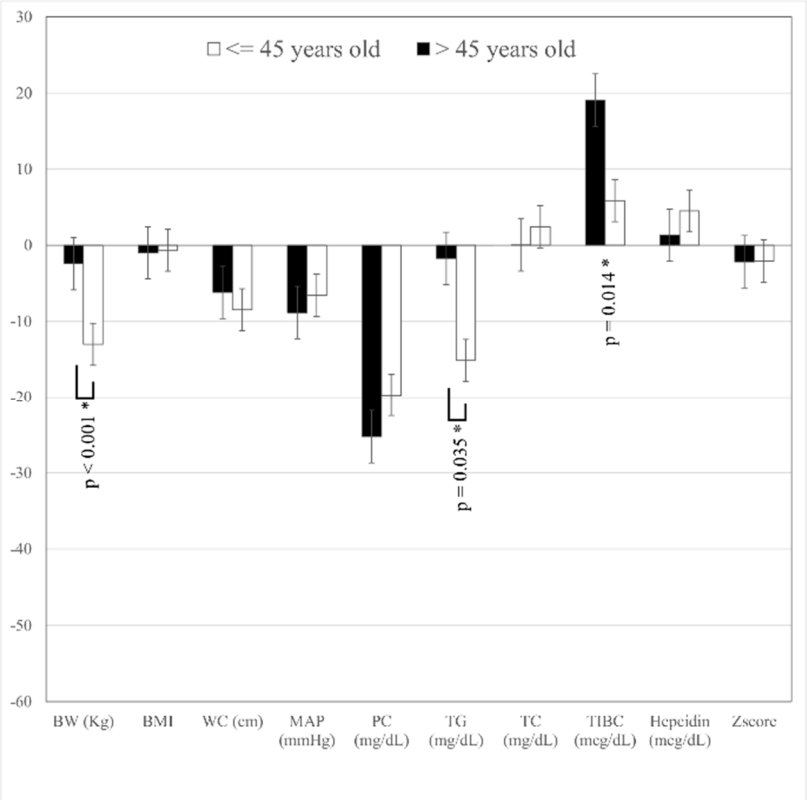
CR



CRF

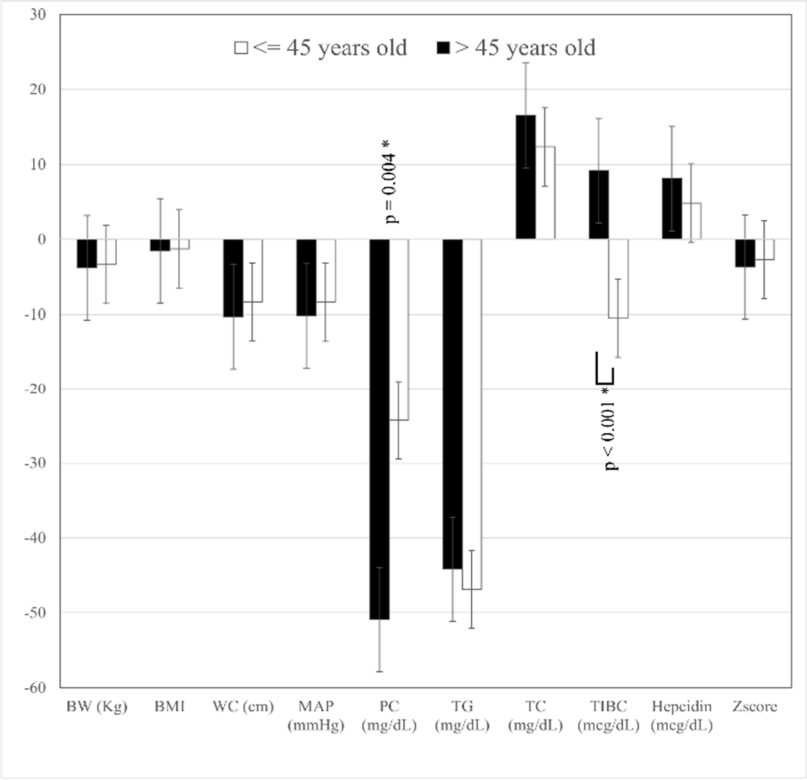


Figure 2. Changes in selected biochemical and clinical characteristics after 12-week dietary intervention in each treatment group. BW, body weight, BMI, body mass index; WC, waist circumference; MAP, mean arterial pressure; PC, postprandial glucose test (2 hour after eating); TG, triglyceride; TC, total cholesterol;; LDL-C, low density lipoprotein cholesterol; TIBC, total iron-

binding capacity. Data are presented as the mean±SEM. *Twelve weeks after intervention is significantly different by independent *t*-test, $p<0.05$

3.5. Correlation between selected variables changes and MetS severity

Table 5 presents the correlations of changes in MetS severity (Z-scores) with selected anthropometric and biochemical variables. The anthropometric changes in BMI and android, gynoid, and total body fat percentages were positively correlated with the changes in MetS severity (r = 0.248 and 0.304, 0.261, 0.331, respectively; all $p < 0.05$). The changes in the lean mass/fat mass ratio were inversely correlated with the changes in MetS severity (r = −0.349, $p < 0.001$). The metabolic changes in the PC and serum TG levels positively correlated with the changes in MetS severity (r = 0.253 and 0.517, respectively; $p < 0.05$).

Table 5. Correlation between changes in Z-scores and selected anthropometric, metabolic, and inflammatory parameters, iron status, and LC-PUFAs

Variables	Change in Z-scores (correlation)	*p*
Anthropometric parameters		
ΔBMI (kg/m²)	0.248	0.003*
ΔAndroid fat (%)	0.304	0.000*
ΔGynoid fat (%)	0.261	0.002*
ΔTotal body fat (%)	0.331	<0.001*
ΔLean/fat ratio	−0.349	<0.001*
Metabolic parameters		
ΔPC (mg/dL)	0.253	0.003*
ΔTG (mg/dL)	0.517	<0.001*
ΔTC (mg/dL)	−0.025	0.775
ΔLDL-C (mg/dL)	−0.011	0.895
Inflammatory parameters		
ΔIL-6 (pg/dL)	0.061	0.482
Iron parameters		
ΔTIBC	−0.047	0.586
LC-PUFAs		
ΔALA	−0.137	0.149
Δn-6/n-3	−0.104	0.275

BMI, body mass index; PC, postprandial glucose test (2 h after eating); HbA1c, haemoglobin A1C; TG, triglyceride; TC, total cholesterol; LDL-C, low density lipoprotein cholesterol; IL-6, interleukin-6. TIBC, total iron-binding capacity; LC-PUFAs, long-chain polyunsaturated fatty acids; AA, arachidonic acid; ALA, alpha-linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; n-6 PUFA, n-6 polyunsaturated fatty acid; n-3 PUFA, n-3 polyunsaturated fatty acid. *$p < 0.05$

4. Discussion

This study showed that CR and CRF interventions reduce the severity of MetS in obese middle-aged women. Furthermore, the selected anthropometric and laboratory profiles returned to normal ranges following the interventions. Nutritional strategies such as CR and changing carbohydrate, protein, and fat consumption are recommended for reducing BW. During a 6-month trial in obese patients, a low carbohydrate diet was associated with BW loss and reduction in cardiovascular risk markers [20]. The dietary plan based on an average restriction of 500 kcal as per the Harris–Benedict formula was used to calculate the BMR in accordance with the United Nations Food and Agriculture Organization and World Health Organization guidelines for physical activity [21]. In this study, the participants consumed a diet of 1,400–1,600 kcal per day for 12 weeks. The diet comprised 53% carbohydrates, 18% animal and plant proteins, and ≤30% fat. This CR regime reduced the BWs of the participants by 5%–10%, which is associated with lower IR and decreased T2D development. Similar

findings were observed in a moderately restricted calorie and carbohydrate diet intervention that showed a positive effect on BW loss and MetS characteristics in overweight and obese participants and prediabetic patients [20].

CRs with manipulation of dietary carbohydrate content have attracted increasing interest because of their effectiveness for BW loss, glycemic control, insulin sensitivity, and cardiovascular risk factor management [22]. CR through reduced carbohydrate content considerably reduced visceral fat mass in Wistar rats, suggesting its potential beneficial effect on fat distribution and IR in humans [23]. The reduction in plasma TG levels caused by carbohydrate-restricted diets results from the downregulation of hepatic de novo lipogenesis [24]. Moreover, carbohydrate restriction increases muscle lipoprotein lipase (LPL) levels, thus enhancing TG clearance. Increasing tissue LPL expression and activity causes the increase in HDL-C levels. Increased LPL-mediated catabolism of TG-rich lipoproteins aids the transfer of unesterified cholesterol, apolipoprotein, and phospholipid during mature HDL-C formation [25].

Studies have reported that CR supplemented with n-3 PUFA can reduce blood glucose and TG levels [23, 26, 27]. However, the individual contributions of proteins, lipids, and reduced calorie intake to these effects have yet to be fully elucidated. Our interventions improved the pre-T2D status of participants with high baseline FBC and PC levels and high HOMA-IR and QUICKI scores. We observed significant reductions in TG levels in the groups treated with n-3 PUFAs, which correlated with the Z-scores for MetS severity. However, the ability of supplementation with n-3 PUFA alone to reduce the severity of cardiometabolic symptoms in MetS patients requires further investigation.

Clinical studies have shown a significant association between the quality and quantity of fat intake and MetS [28, 29]. Several clinical trials supplemented CR with higher amounts of n-3 PUFAs (1–3 g) and demonstrated TG-lowering effects over a long term period (6 months), which may be attributable to declined liver TG secretion enhancing plasma TG clearance, improving antioxidative enzymes status, and removing chronic pro-inflammatory markers [28–31]. A number of well-supported reviews have indicated that n-3 PUFA consumption and chronic disease prevention are associated [32, 33]. Major metabolic stressors including energy-dense and high-fat diets were shown to promote obesity, IR, and MetS [33]. Abnormal tissue and organ dysfunction in MetS patients may cause chronic inflammation. Studies have reported that the increased dietary intake of n-3 PUFAs alters the fatty acid composition of membrane phospholipids and reduces the serum n-6/n-3 PUFA ratio and pro-inflammatory eicosanoid production [34, 35]. In this 12-week trial, we administered 2,130 mg of fish oil (1,280 mg of EPA and 850 mg of DHA) to obese middle-aged women and observed reduction in the levels of the chronic pro-inflammatory markers CRP and IL-6. The mechanisms may be attributable to n-3 PUFA being a modulator of the activation of serine or threonine phosphorylation cascades that cause the activation of nuclear factor-κB, as well as to the serine phosphorylation of insulin receptor signalling system elements in selected organs and tissues (i.e. liver and muscle) [36]. By contrast, the increased intake of n-3 PUFAs, particularly EPA and DHA, may contribute to the production of eicosanoids with lower inflammatory potential than those of AA analogues [37]. The n-6/n-3 ratio in RBCs may more reliably indicate the chronic inflammatory status of abnormal metabolic processes than PUFA metabolite levels [36].

Increased hepatic iron storage elevates T2D risk factors [38]. Oxidative stress, cell toxicity, and genotoxicity increase iron deposits resulting from excessive circulatory iron levels. Studies have suggested that reducing the liver's capacity for insulin extraction results in impaired suppression of hepatic glucose production, as well as the retardation of glucose disposal in adipocytes and muscle cells [39, 40]. In this study, the results of iron serum status in obese MetS participants did not change after intervention. After the 12-week CR and CRF interventions, although serum iron levels remained unchanged, the TIBC decreased significantly. Thus, we inferred that our participants had an unsaturated serum iron status before intervention. The serum iron levels and TIBC after both interventions revealed an effective maintenance of unsaturated iron binding capacity in our participants.

In this study, CRF more effectively reduced the biochemical and clinical variables of MetS (particularly the TG, TC, IL-6, BMI, total body fat level and Z-score) in older middle-aged women

than in younger middle-aged women. The main factors causing MetS in the middle-aged participants were physical inactivity and a high-fat and -carbohydrate diet, both of which contributed to the two main clinical features of MetS, namely central obesity and IR [41]. Because obesity appears to precede the emergence of the other MetS risk factors, it is necessary for MetS prognosis. This study showed that a 12-week CRF intervention effectively reduced BW and other major MetS characteristics, thus reducing MetS severity in the middle-aged female participants.

Some limitations to this study must be noted. First, our study focused on the effects of fish oil supplementation on MetS-related variables. However, we did not evaluate its effects on other nutritional parameters, such as urinary ketones, nitrogen balance, bone health, and muscle activity. Second, the outcomes of self-administered calorie-restricted dietary treatments can be inconsistent. The participants' food-selecting behaviours could have been potential confounding factors in this study; nevertheless, our well-designed self-monitoring programme and regular group meetings provided assistance to participants encountering difficulties in understanding the dietary regime. Third, although we provided participants with official dietary guidelines, some participants did not strictly adhere to the suggested dose of 2 g of fish oil during the trial period and some participants demonstrated poor compliance during the festival season. Fourth, although this study showed significant amelioration of MetS severity in middle-aged women, the gender effect may have caused an overestimation of the effects of CR on BW loss. Further research on dietary intervention for MetS patients is necessary to improve treatments.

5. Conclusion

The study findings indicate that CR and CRF dietary interventions improve the anthropometric and MetS characteristics of middle-aged women, including their BW, blood glucose and TG levels, HOMA-IR and QUICKI scores, and MetS severity. Our dietary interventions were more effective in older middle-aged women (>45 years) than in younger middle-aged women (≤45 years).

Conflict of Interest: Dr Shih-Yi Huang received consulting and lecture fees from Herbalife Taiwan Co., Ltd. No potential conflicts of interest are reported by the other authors.

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