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ORIGINAL ARTICLE

A calorie-restriction diet supplemented with fish oil and high-protein powder is associated with reduced severity of metabolic syndrome in obese women

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BACKGROUND/OBJECTIVES: The prevalence of metabolic syndrome (MetS) and obesity has increased worldwide, as well as in Taiwan, particularly in women aged > 40 years. The purpose of this study was to elucidate the effects of a calorie-restriction diet (CR) supplemented with protein and n-3 polyunsaturated fatty acids (PUFAs) on women with MetS.

SUBJECTS/METHODS: A total of 143 eligible female participants were recruited and assigned to four dietary interventions such as 1500-kcal CR, calorie-restriction meal-replacement diet (CRMR), calorie-restriction diet with fish oil supplementation (CRF) and calorie-restriction meal-replacement diet with fish oil supplementation (CRMRF). The changes in anthropometric measures, metabolic profiles, inflammatory response and the Z-score of severity of MetS were evaluated.

RESULTS: Among 143 female MetS patients enrolled, 136 patients completed the 12-week study. After the 12-week dietary interventions, we observed reductions in body weight (BW), body mass index (BMI) and waist circumference (WC) in all groups. BMI and triglyceride (TG) levels decreased significantly in the CRMR, CRF and CRMRF groups, but not in the CR group. The homeostasis model assessment of insulin resistance (HOMA-IR) had significantly improved in all four groups, and the levels of interleukin-6 (IL-6) and C-reactive protein (CRP) had significantly decreased in the CRF and CRMRF groups. Following the interventions, the changes in waist circumference (WC), mean arterial pressure (MAP), fasting blood glucose (FBG), TGs, HOMA-IR, CRP and IL-6 significantly correlated with the reductions in Z-score of MetS severity.

CONCLUSIONS: Our study results indicate that a calorie-restriction dietary intervention combined with various macronutrients can reduce the severity of MetS in women and increase recovery from MetS by almost twofold in comparison with a CR alone.

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INTRODUCTION

The prevalence of metabolic syndrome (MetS) has increased worldwide, in parallel with an increase in the rates of obesity.[1] Such trends have also occurred in Taiwan, particularly in women aged > 40 years.[2] MetS is a cluster of conditions, including excess weight, insulin resistance, dyslipidemia and hypertension, which act multiplicatively to promote cardiovascular disease.[3] Lifestyle modifications are recommended as the first priority for managing MetS.[4,5]

Clinical trials have reported that changing the macronutrient quality of a diet can promote weight loss and increase insulin sensitivity.[6–8] Studies have also demonstrated that restricting dietary calories[6] and increasing intake of certain macronutrients,[7,8] particularly n-3 long-chain polyunsaturated fatty acids (PUFAs), can benefit patients with metabolism-related disorders. Interventional studies with calorie-restriction diets (CR) have reported that partially increasing dietary protein[9,10] or certain lipids (n-3 PUFAs)[11] can reduce hyperlipidemia and hypertension in patients with MetS. A prospective epidemiological study further indicated that consumption of fish might prevent the development of insulin resistance.[12] However, methods of increasing protein intake without increasing energy intake should also be considered.[13,14]

Previous studies have identified that overweight and obese patients tend to be associated with a high inflammatory index and abnormal glycemic response.[15,16] In patients with MetS, chronic inflammatory status is attributed to high-calorie diets and inadequate lipid profiles (that is, high levels of saturated n-6 PUFAs and less than optimal levels of n-3 PUFAs).[17] Therefore, the investigation of alternative dietary fatty acid profiles might facilitate the development of strategies for the prevention of chronic diseases.

Storlien *et al.*[18] identified that n-3 PUFAs, particularly eicosapentaenoic acid (C20:5) and docosahexaenoic acid (C22:6), increase insulin sensitivity in animal models. Studies have also shown that a low-calorie diet with n-3 PUFA supplementation can reduce body fat and promote weight loss.[17,19] However, data are inconsistent on the relationship between the consumption of n-3 PUFAs and fish and MetS.[20] Therefore, the purpose of this study was to elucidate the effects of a CR supplemented with different macronutrients, such as protein, n-3 PUFAs and their combination, on women with MetS. Specifically, we aimed to evaluate the effects of the different nutrients on anthropometric measures, metabolic profiles and inflammatory cytokines in women with MetS.

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MATERIALS AND METHODS

Participants

Eligible female participants ($n = 143$) were recruited from May 2012 to March 2013 based on the following criteria: body mass index (BMI) $\geqslant 24$ and $\leqslant 35\ kg/m^2$, waist circumference (WC) $\geqslant$ 80 cm and $\geqslant 2$ MetS signs according to the Taiwanese MetS criteria of low high-density lipoprotein cholesterol (HDL-C) (< 50 mg/dl in women), hypertension (blood pressure (BP) $\geqslant 135/85$ mm Hg), high blood glucose (blood glucose > 100 mg/dl) and hypertriglyceridemia (triglycerides (TGs) $\geqslant$ 150 mg/dl). Patients were excluded if they had diabetes, coronary heart disease, cardiovascular disease, neoplastic, renal, liver, endocrine or psychiatric diseases, were smokers, consumed excess alcohol, were pregnant or planning a pregnancy or were sensitive or allergic to fish. Patients were also excluded if they had taken any lipid-lowering agent, antihypertension or hypoglycemic medication, or n-3 PUFA supplement. The patients were recruited through local community advertisements, and the study was conducted at the School of Nutrition and Health Science, Taipei Medical University and its affiliated hospital in Taipei, Taiwan. This study was approved by the Joint Institutional Review Board in Taipei Medical University and registered at ClinicalTrials.gov as NCT01768169.

Study design and procedures

A randomized parallel-arm open controlled study was designed to evaluate the effects of four dietary interventions on women with MetS. Patients were randomly allocated one of four dietary interventions: calorie-restriction diet (CR), calorie-restriction meal-replacement diet (CRMR), calorie-restriction diet with fish oil supplementation (CRF) and calorie-restriction meal-replacement diet with fish oil supplementation (CRMRF). Table 1 lists the nutritional compositions of the 150- kcal diets.

Patients were asked to provide informed consent before random assignment into the four groups. At 0, 6 and 12 weeks, they received anthropometric, biochemical, clinical and dietary assessments. The patients also received a group teaching program, which included 1-h weekly classes on a healthy diet, determining the fat content in foods, skills for eating out, behavioral changes and exercise. Patients were asked to maintain daily dietary records, which were evaluated by a registered dietician on a weekly basis.

Calorie-restriction dietary intervention

A registered dietician estimated the patients' total daily energy requirements to determine individual requirements at the baseline. The basal metabolic rate was calculated using the Harris–Benedict equation[21] for women, which is as follows: $655.1 + 9.6 \times$ (weight in kg) $+ 1.9 \times$ (height in cm) $- 4.7 \times$ (age in years). The basal metabolic rate was then multiplied by 1.3 to estimate the total energy requirements. An energy deficiency of 500–800 calorie/day was applied to the total energy requirements of each patient. The macronutrient distribution of the total calories was based on the Taiwanese dietary guidelines of 2011, with 50–55% from carbohydrates, 15–20% from proteins, $< 30\%$ from fat and recommended cholesterol intake of < 300 mg/day.

Participants in the meal-replacement groups received a diet plan in which dinner was substituted by 25 g of a high-protein meal replacement (Low Calorie Nutrition Drink Mix powder; Herbalife, Los Angeles, CA, USA), which provides 8 g of protein, 0.6 g of fat, 11 g of carbohydrates and 81 kcal/serving. In the fish oil-supplemented groups, all participants were instructed to consume 10 capsules of fish oil (Herbalifeline; Herbalife) per day, which provided 2130 mg of n-3 PUFAs (1280 mg of eicosapentaenoic acid and 850 mg of docosahexaenoic acid). Compliance with meal replacement and fish oil supplementation was assessed by calculating the weight of the leftover meal replacement, and counting the number of fish oil capsules returned every 2 weeks.

Anthropometric measurements and body composition

Patient body height was measured to the nearest 0.5 cm by using a wall-mounted stadiometer, with the patient barefoot, and body weight (BW) was measured to the nearest 0.1 kg by using a TANITA (SC-330, Tokyo, Japan) electronic scale, with the patient wearing light clothing and no shoes. At each visit, anthropometric assessments and body fat percentage were measured by using a bioimpedance device (InBody 720, Biospace, Seoul, Korea). Body composition was estimated using a dual-energy X-ray absorptiometer (Lunar Prodigy, GE Healthcare, Madison, WI, USA). Android and gynoid fat distribution was measured from a dual-energy X-ray absorptiometer scan obtained at an umbilical level in the supine position. The dual-energy X-ray absorptiometer scan data were analyzed using the Lunar enCORE 2006 software, Version 10.50.086 (GE Healthcare).

Clinical and biochemical assessments

BP was measured in a sitting position from the right arm after a 5-min rest by using an automatic sphygmomanometer (Omron HEM-781INT, Kyoto, Japan). Blood samples were collected at baseline, and at weeks 6 and 12, through a catheter in an antecubital vein after a 12-h overnight fast. A 2-h oral glucose tolerance test was conducted after overnight fasting, and blood glucose and serum insulin levels were determined 0 and 120 min after consuming 75 g of glucose. Blood samples were maintained at $-80\ ^\circ C$ until analysis. Serum albumin, total protein, TGs and cholesterol were measured using an automated analyzer (Ortho Clinical Diagnostics VITROS 950, Johnson & Johnson, New Brunswick, NJ, USA). Serum low-density lipoprotein-cholesterol (LDL-C) and HDL-C were analyzed using an automated analyzer (Toshiba TBA-c16000, Toshiba, Tokyo, Japan). Serum insulin was analyzed using a radioimmunoassay kit (DIA Source, Lovain-La-Nueve, Belgium). Blood glucose was determined using an automated analyzer (VITOR 5, 1FS, Ortho Clinical Diagnostics, Johnson & Johnson) with Victors Chemistry Products GLU slides by using a hexokinase method. Hemoglobin (Hb)A1c was determined using an HLC-723 GHb G7 analyzer (Tosoh, Tokyo, Japan). Serum C-reactive protein (CRP) concentrations were analyzed using an automated analyzer (Toshiba TBA-c16000), and interleukin (IL)-6 was measured using a Qunatikine high-sensitivity commercial enzyme-linked immunosorbent assay kit (R&D Systems, Minneapolis, MN, USA).

Assessment of insulin resistance

The homeostasis model assessment of insulin resistance (HOMA-IR) was used as an indicator of insulin resistance, evaluated using a glucose clamp technique. Insulin and glucose values were obtained to calculate the HOMA-IR using the formula HOMA-IR = (fasting plasma insulin (μIU/mol) × fasting plasma glucose (mol/l)/22.5. The quantitative insulin sensitivity check index (QUICKI), derived from fasting blood glucose (FBG) and insulin levels, was also used as an index of insulin resistance: QUICKI[22] = 1/(log

Table 1. Nutritional compositions of the calorie-restriction diets (1500 kcal)

Groups	*CR*	*CRMR*	*CRF*	*CRMRF*
Calories (kcal)	1483	1513	1528	1558
Carbohydrates (g) (%)	192 (51.8%)	203 (53.7%)	192 (50.3%)	203 (52.1%)
Protein (g) (%)	64 (17.3%)	57 (15.1%)	64 (16.8%)	57 (14.6%)
Protein from meal replacement (g) (%)		8 (2.1%)		8 (2.1%)
Fat (g) (%)				
Saturated fatty acids (g) (%)	21 (12.7%)	19 (11.3%)	21 (12.4%)	19 (11.0%)
PUFAs (g) (%)	30 (18.2%)	30 (17.89%)	30 (17.6%)	30 (17.3%)
n-3 PUFAs (g) (%)			5 (2.9%)	5 (2.9%)

Abbreviations: CR, calorie-restriction diet; CRF, calorie-restriction diet with fish oil supplementation; CRMR, calorie-restriction meal-replacement diet; CRMRF, calorie-restriction meal-replacement diet with fish oil supplementation; PUFA, polyunsaturated fatty acids.

(fasting insulin μU/ml)+log (fasting glucose mg/dl)). The Z-score is defined by five MetS criteria and provides an indicator of MetS severity.[23] The equation used to calculate the Z-score was as follows: $Z\text{-score}_{women} = ((50 - HDL)/12.4)+((TGs - 150)/66.5)+((FPG - 100)/13.4)+((WC - 88)/11.7) +((\text{mean arterial pressure (MAP)} - 100)/10.03)$.

Statistical analyses

An analysis of variance was used to test the differences among groups at baseline. Differences in baseline characteristics between the treatment and control groups were assessed using Student's *t*-tests by using SAS 9.1.3 with Service Pack 3 (SAS Institute, Chicago, IL, USA). To examine the differences among the four groups following the interventions, a one-way analysis of variance was applied. A Pearson's correlation was used to examine the associations among outcomes.

RESULTS

Among 143 female MetS patients enrolled, 136 patients completed the 12-week study. Seven patients withdrew from the study because of personal issues ($n = 4$), family reasons ($n = 1$), relocation ($n = 1$) and change of mind ($n = 2$). The dropout rate for the trial was 4.9%. Figure 1 shows a flowchart diagram of participant selection. The basic patient characteristics exhibited non-significant differences among the four groups.

Anthropometric and clinical characteristics

After the 12-week dietary interventions, we observed reductions in BW, BMI and WC in all four patient groups (Table 2). The range of weight reduction was 2.3–6.2 kg. BMI decreased significantly in the CRMR ($P = 0.045$), CRF ($P = 0.047$) and CRMRF ($P = 0.047$) groups, but not in the CR group. WC decreased significantly in all groups in the range of 6.5–9.6 cm. We observed significant reductions in % android fat in the CRMRF group ($P < 0.05$) and significant reductions in % gynoid fat in the CRMR group ($P < 0.05$). We observed a trend toward increased lean mass ratio in each group post intervention, although this trend was non-significant.

Effects of the dietary interventions on glycemic, lipid and inflammatory parameters

After the 12-week dietary interventions, FBG and postprandial glucose (PC) had significantly decreased in the CRF and CRMRF groups ($P < 0.05$) (Table 3). In all four groups, the fasting insulin levels had significantly decreased ($P < 0.05$). The TG levels also had significantly decreased in the CRMR, CRF and CRMRF groups, but not in the CR group. Post intervention, we observed significantly reduced LDL-C levels (138.1 ± 35.9 vs 117.3 ± 31.8 mg/dl, $P = 0.012$) in the CRMRF group, and significantly reduced HDL-C levels (54.6 ± 10.9 vs 48.0 ± 9.2 mg/dl, $P = 0.014$) in the CRMR group (Table 3). The HOMA-IR had significantly improved in all four groups ($P < 0.05$), and the levels of IL-6 and

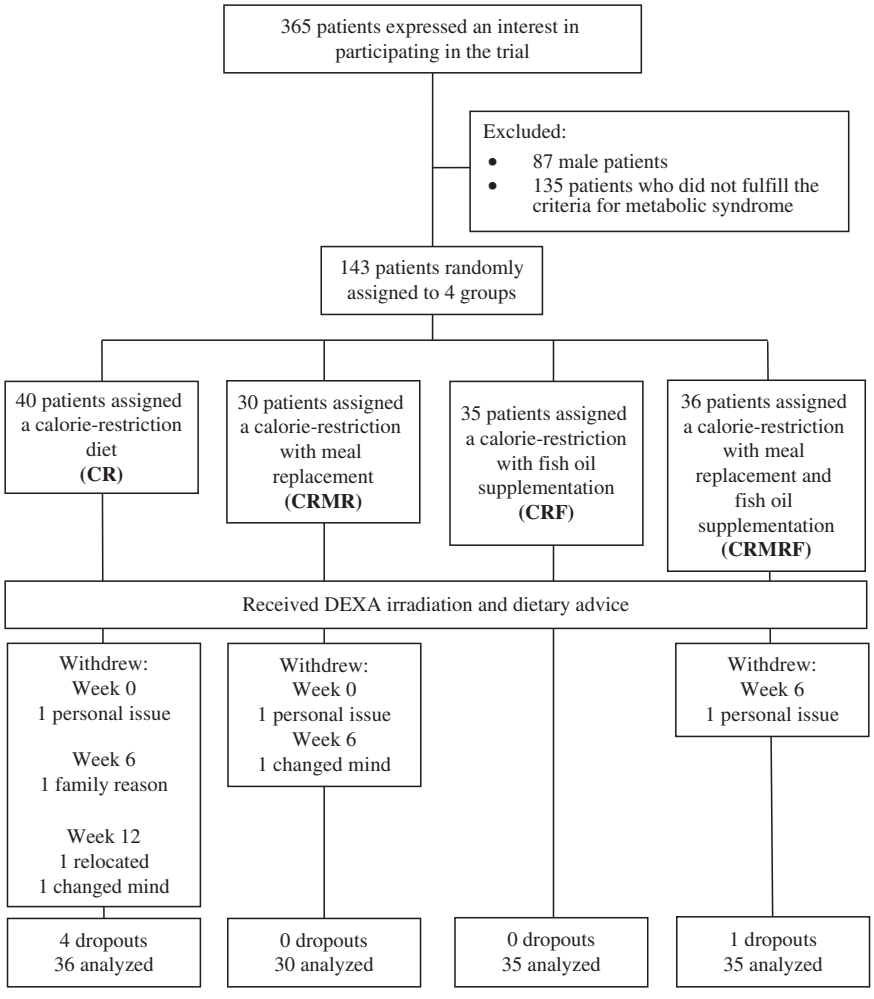

Figure 1. Flowchart diagram of participant selection. Participants were assigned to one of the following dietary intervention groups: CR, CRMR, CRF or CRMRF for 12 weeks of treatment. In total, 136 participants completed the study.

Table 2. Selected anthropometric characteristics of participants after the 12-week dietary interventions

		Body weight (kg)	*BMI (kg/m²)*	*WC (cm)*	*MAP (mmHg)*	*Android fat (%)*	*Gynoid fat (%)*	*Total body fat (%)*	*Lean mass ratio*
CR ($n=36$)	Baseline	75.0 ± 12.4	29.9 ± 4.6	92.0 ± 9.2	104.7 ± 11.3	50.4 ± 4.1	45.3 ± 4.8	42.2 ± 4.8	1.40 ± 0.28
	Week 12	72.7 ± 12.7	29.0 ± 4.7	86.7 ± 8.8*	96.0 ± 10.4*	49.4 ± 5.0	44.6 ± 5.2	41.4 ± 5.3	1.45 ± 0.32
CRMR ($n=30$)	Baseline	76.1 ± 13.9	29.5 ± 4.6	88.2 ± 7.4	102.1 ± 8.4	50.5 ± 5.3	47.5 ± 5.3	43.4 ± 5.7	1.36 ± 0.33
	Week 12	69.9 ± 13.8	27.0 ± 4.6*	83.4 ± 8.6*	92.0 ± 10.8*	47.7 ± 6.6	44.4 ± 6.4*	40.5 ± 7.0	1.55 ± 0.47
CRF ($n=35$)	Baseline	73.6 ± 11.2	29.3 ± 3.1	90.8 ± 8.1	103.9 ± 10.8	50.5 ± 5.1	46.3 ± 5.5	42.8 ± 5.4	1.37 ± 0.30
	Week 12	69.9 ± 10.7	27.8 ± 2.9*	82.8 ± 7.9*	94.7 ± 11.3*	48.7 ± 5.2	45.1 ± 5.3	41.3 ± 5.3	1.43 ± 0.36
CRMRF ($n=35$)	Baseline	71.1 ± 12.6	28.6 ± 3.9	88.2 ± 8.4	106.0 ± 11.1	49.7 ± 5.0	44.8 ± 6.9	41.4 ± 6.1	1.46 ± 0.34
	Week 12	66.3 ± 12.0	26.7 ± 3.9*	83.4 ± 8.6*	95.0 ± 12.2*	46.7 ± 5.8*	42.4 ± 6.7	38.7 ± 6.8	1.66 ± 0.45

Abbreviations: BMI, body mass index; CR, calorie-restriction diet; CRF, calorie-restriction diet with fish oil supplementation; CRMR, calorie-restriction meal-replacement diet; CRMRF, calorie-restriction meal-replacement diet with fish oil supplementation; MAP, mean arterial pressure = (2 × systolic blood pressure +diastolic blood pressure)/3; WC, waist circumference. Data are presented as the mean ± s.d. *$P > 0.05$.

Table 3. Selected biochemical characteristics of participants after the 12-week dietary interventions

		Fasting blood glucose (mg/dl)	*PC (mg/dl)*	*Insulin (μIU/ml)*	*HOMA-IR*	*Triglycerides (mg/dl)*	*LDL-C (mg/dl)*	*HDL-C (mg/dl)*	*Z-score*
CR ($n=36$)	Baseline	106.0 ± 23.8	148.3 ± 58.8	17.5 ± 8.0	4.6 ± 2.2	137.1 ± 93.6	130.4 ± 28.4	50.0 ± 8.0	1.5 ± 0.5
	Week 12	101.5 ± 16.7	123.2 ± 47.3	11.6 ± 5.9*	2.9 ± 1.6*	144.5 ± 82.3	112.9 ± 32.0	49.5 ± 8.5	− 1.0 ± 0.7*
CRMR ($n=30$)	Baseline	96.2 ± 7.6	143.9 ± 35.3	17.6 ± 9.1	4.2 ± 2.2	132.3 ± 53.4	133.5 ± 33.5	54.6 ± 10.9	1.2 ± 0.2
	Week 12	95.2 ± 6.8	122.1 ± 26.9	11.2 ± 5.1*	2.7 ± 1.3*	106.5 ± 43.3*	122.7 ± 27.2	48.0 ± 9.2*	− 2.0 ± 0.4*
CRF ($n=35$)	Baseline	101.2 ± 10.2	141.7 ± 43.6	14.2 ± 5.9	3.5 ± 1.4	135.8 ± 53.3	132.8 ± 39.3	51.7 ± 11.6	1.0 ± 0.3
	Week 12	94.4 ± 6.6*	99.9 ± 24.8*	11.3 ± 4.3*	2.7 ± 1.1*	113.0 ± 33.3*	131.3 ± 32.0	52.7 ± 11.4	− 2.1 ± 0.3*
CRMRF ($n=35$)	Baseline	104.0 ± 13.3	149.5 ± 53.5	17.2 ± 11.8	4.4 ± 3.2	132.1 ± 58.3	138.1 ± 35.9	50.2 ± 8.7	1.1 ± 0.3
	Week 12	94.9 ± 9.9*	119.5 ± 30.8*	11.6 ± 7.0*	2.7 ± 1.7*	108.6 ± 44.9*	117.3 ± 31.8*	50.1 ± 10.5	− 1.9 ± 0.4*

Abbreviations: CR, calorie-restriction diet; CRF, calorie-restriction diet with fish oil supplementation; CRMR, calorie-restriction meal-replacement diet; CRMRF, calorie-restriction meal-replacement diet with fish oil supplementation; FBG, fasting blood glucose; HDL-C, high-density lipoprotein cholesterol; HOMA, homeostatic model assessment; LDL-C, low-density lipoprotein cholesterol; MAP, mean arterial pressure; PC, postprandial glucose; TG, triglyceride; WC, waist circumference. Data are presented as the mean ± s.d. *$P > 0.05$. Female Z-score = ((50 − HDL-C)/12.4)+((TG − 150)/66.5)+((AC − 100)/13.4)+((WC − 88)/11.7) +((MAP − 100)/10.03).

CRP had significantly decreased in the CRF and CRMRF groups (Figure 2).

Following the 12-week dietary interventions, the Z-score significantly decreased in all four groups ($P < 0.05$). The MetS recovery rates in the CR, CRMR, CRF and CRMRF groups were 38.9% (14/36), 66.7% (20/30), 71.4% (25/35) and 62.9% (22/35), respectively. The factor demonstrating highest recovery post intervention was the level of TGs, and the factor associated with lowest recovery was HDL-C.

Correlations between changes in Z-score and MetS criteria and inflammatory factors

Table 4 lists the correlations between the changes in Z-score and the anthropometric, metabolic and inflammatory parameters. The changes in WC, MAP, FBG, TGs, HOMA-IR, CRP and IL-6 significantly correlated with the reductions in MetS severity (Z-score). The changes in the CRP levels significantly correlated with the changes in AC ($r = 0.177$, $P = 0.039$). The changes in IL-6 significantly correlated with the changes in the WC, MAP, % android fat, % gynoid fat, total body fat %, lean mass ratio, FBG and TGs.

DISCUSSION

In this study, we observed that a 12-week CR, supplemented with high-protein meal-replacement powder and bioactive ingredients, such as n-3 PUFAs, can improve the metabolic profiles and inflammatory cytokine levels of women with MetS. A CR supplemented with high-protein powder and n-3 PUFAs results in 1.5-fold greater recovery from MetS (as indicated by Z-score) in comparison with a CR alone. Our study results indicate that meal replacement, alone or in combination with n-3 PUFA supplementation, achieves greater weight loss in comparison with a CR alone, although the differences are not statistically significant.

After 12 weeks of intervention, our MetS patients with CR with n-3 PUFA supplementation and/or meal replacement were associated with significantly reduced fasting blood glucose, insulin and oral glucose tolerance test 2-h blood glucose. The n-3 PUFAs might have contributed to improve glucose metabolism; however, data from previous studies on the relationship between n-3 PUFAs and glucose metabolism provided inconclusive results. Epidemiological studies on the associations between n-3 PUFAs and risk of type 2 diabetes mellitus provided inconclusive results.[24,25] Dangardt *et al.*[26] observed that n-3 PUFA supplementation affected the fatty acid composition of muscle, and glucose and

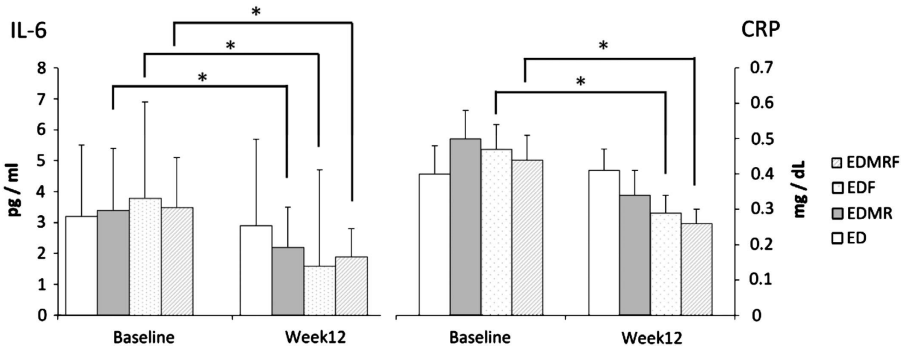

Figure 2. Inflammatory factor levels of participants after the 12-week dietary interventions. Data are presented as the mean ± s.d. *$P > 0.05$.

Table 4. Correlations between changes in Z-scores and anthropometric, metabolic and inflammatory parameters

Variable	*Change in Z-score correlation*	P
Anthropometric parameters		
Body weight	0.111	0.197
Waist circumference	0.383	< 0.001
Android fat (%)	0.301	0.001
Gynoid fat (%)	0.274	0.001
Body fat (%)	0.331	< 0.001
Lean mass ratio	0.353	< 0.001
Metabolic parameters		
Mean arterial pressure	0.682	< 0.001
FBG	0.589	< 0.001
PC	0.233	0.006
Hemoglobin A1c	0.110	0.203
Insulin	0.147	0.088
Triglycerides	0.511	< 0.001
Low-density lipoprotein cholesterol	−0.011	0.894
High-density lipoprotein cholesterol	−0.346	0.004
Glycemic parameters		
Homeostatic model assessment of insulin resistance	0.227	0.008
Qualitative insulin sensitivity check index	−0.398	< 0.001
Inflammatory parameters		
Interleukin-6	0.558	< 0.001
C-reactive protein	0.200	0.020

Abbreviation: FBG, fasting blood glucose.

insulin homeostasis in obese young participants. Our data suggest that n-3 PUFAs have a role in insulin and glucose metabolism in women with MetS.

Previous studies have demonstrated the lowering effects of n-3 PUFAs on TG levels, identifying that 3–4 g/day of n-3 PUFAs reduces plasma TGs by approximately 30%.[27] In this study, we instructed the patients in the two fish oil-supplemented groups to consume 10 capsules of fish oil per day, which provided 2130 mg of n-3 PUFAs (1280 mg of eicosapentaenoic acid and 850 mg of docosahexaenoic acid) and resulted in reduced TG levels (by 17%). The CR with meal replacement produced the greatest weight loss and % weight change, and significantly reduced TG levels by approximately 20%. The meal-replacement group without n-3 PUFA supplementation was also associated with significantly reduced TG levels. This result indicates that weight reduction (> 8% of the original BW) without fish oil supplementation can exert beneficial effects on women with MetS. The effects of n-3 PUFAs are dose dependent, with a reported minimal effective dose of > 2 g/day.[28] The dose of n-3 PUFAs provided in our study exceeded the minimal effective dose.

A previous meta-analysis investigated the long-term effects of CR and observed that high-protein and low-protein diets did not reduce total cholesterol or LDL-C levels.[29] In our study, we observed a trend for reduced LDL-C in all four dietary intervention groups; however, the trend was only significant in the meal-replacement with n-3 PUFA group. This result indicates that weight reduction alone cannot improve LDL-C levels unless supplemented with n-3 PUFAs. Although we observed significantly reduced HDL-C levels in the group with largest weight loss (CRMR), the 2 n-3 PUFA-supplemented groups were not associated with reduced HDL-C levels. Our observations of the effects of a CR on HDL-C levels were similar to those in a previous study in which weight reduction in overweight women modified atherogenic dyslipidemia,[30] causing reduced HDL-C.

Women with MetS have higher high-sensitivity CRP (hs-CRP) levels than do women without MetS.[31] Our patients with MetS exhibited high hs-CRP levels (> 0.3 mg/dl), which were associated with non-significant reductions after the calorie-restriction (< 30% fat) and calorie-restriction with meal-replacement dietary interventions. However, in the dietary groups with n-3 PUFA supplementation, we observed the significant reduction in hs-CRP levels post intervention (< 0.3 mg/dl). Our patients were of Oriental race, and were younger and had lower BMIs than the MetS patients of previous studies did.[31,32] We randomly allocated our patients to four dietary groups, observing that the CR and low-fat diets did not improve their inflammatory conditions. However, the CR with n-3 PUFA supplementation was associated with reduced hs-CRP levels. Although we are unable to establish whether the reduced hs-CRP levels were caused by calorie reduction, fat, protein, n-3 PUFAs or a combination of these factors, our results indicate that adding n-3 PUFAs to a CR can reduce CRP levels in women with MetS of Oriental race.

A proinflammatory status is one of the characteristics of MetS, and hs-CRP and IL-6 are commonly used as inflammatory markers.[33] Esposito *et al.*[34] conducted a randomized single-blind trial using a multidisciplinary program to reduce BW in obese women, and observed reductions in CRP, IL-6 and IL-18. In our study, a CR to reduce BW (< 2.3 kg) did not reduce IL-6 levels, but a CR with n-3 PUFA supplementation reduced both CRP and IL-6 levels. The analyzed inflammatory markers in this study were CRP and IL-6 only. Additional studies analyzing a greater number of sensitive markers in MetS patients are warranted.

Our results and those of previous studies indicate that weight reduction exerts markedly beneficial effects on MetS parameters. In Mason *et al.*,[35] diet- and exercise-induced weight loss improved measures of insulin sensitivity (HOMA and QUICKI). Our study

results indicate that weight reduction by a CR significantly increases insulin sensitivity; however, changes in HOMA and QUICKI values were non-significantly associated with weight loss. Following short-term dietary interventions and exercise, weight loss was also non-significantly associated with increased insulin sensitivity. Clinical trials of longer duration (more than 12 weeks) evaluating various dietary interventions and macronutrient compositions are required to confirm our results.

A study of obese men with high MetS prevalence identified that weight loss of 4.8 kg reduced the odds ratio of MetS by 71% after 2.5 years of follow-up.[36] In our study, the control (CR) group exhibited a MetS recovery rate of 38.9% post intervention. We observed that the addition of n-3 PUFAs to a CR is associated with increased MetS recovery. The MetS factor associated with greatest recovery post intervention was TG levels. It is possible that participants in the ED group could have adopted some additional dietary changes (for example, high-protein powder or n-3 PUFA supplementation) or exercise habits;[37] however, these data are unavailable.

In our study, all dietary groups were associated with significantly reduced HOMA-IR and QUICKI scores. The Z-score used in this study is a continuous score of five MetS variables. Weight reduction significantly improved the MetS Z-score, and changes in inflammatory factors, such as CRP and IL-6, were significantly associated with the changes in the Z-score. Our study is the first to identify a positive correlation between changes in Z-scores and inflammatory factors. Additional studies are required to fully elucidate the association between inflammatory factors and Z-scores in obese women.

There were some limitations that must be mentioned. First, although we provided participants with official dietary guidelines from a registered dietician, some participants did not strictly adhere to the suggested dose of 2 g of fish oil supplement or one meal replacement per day. Second, the trial period included the Chinese New Year festival. Although the meals were supplied and controlled by the Department of Dietetics at TMUH, compliance with meal intake was poor during the festival, and some participants consumed the fish oil supplement but refused the meal replacement. Third, our study focused on the effects of protein-rich meal replacements and long chain-PUFA supplementation on MetS-related variables. However, we did not evaluate the effects of the various dietary treatments on other nutritional parameters such as urinary ketones, nitrogen balance or bone health, or the effects of physical activity on glucose tolerance.

In conclusion, our study results indicate that a calorie-restriction dietary intervention combined with various nutrients can reduce the severity of MetS in women. A CR supplemented with high-protein powder and n-3 PUFAs can increase recovery from MetS by approximately twofold in comparison with a CR alone.

CONFLICT OF INTEREST

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