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Effects of calorie restriction with n-3 long-chain polyunsaturated fatty acids on metabolic syndrome severity in obese subjects: A randomize-controlled trial

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ABSTRACT

Metabolic syndrome (MetS) increases the risk of type 2 DM and cardiovascular disease. We here evaluated the effects of a calorie-restriction diet with 2.13 g/day n-3 long-chain polyunsaturated fatty acid supplementation in MetS patients. The 188 participants were randomly assigned one of four calorie-restriction diets for 12 weeks: calorie-restriction (n = 44), calorie-restriction meal replacement (n = 45), calorie-restriction with fish oil (n = 44), or calorie-restriction meal replacement with fish oil (n = 44); 179 participants completed the trial. After treatment, all groups had significant reduction in waist circumference, blood pressure, and cardiometabolic parameters. Results indicate that long-chain polyunsaturated fatty acid (LC-PUFA) supplementation under calorie-restriction exert beneficial effects on metabolic parameters.

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Abbreviations: MetS, metabolic syndrome; T2DM, type 2 diabetes mellitus; LC-PUFA, long-chain polyunsaturated fatty acid; hsCRP, high-sensitivity C-reactive protein; TMU, Taipei Medical University; TMUH, Taipei Medical University Hospital; WFH, Wan Fang Hospital; BMI, body mass index; WC, waist circumference; TG, triacylglycerol; FBG, fasting blood glucose; SBP, systolic blood pressure; DBP, diastolic blood pressure; AIDS, acquired immunodeficiency syndrome; CR, calorie-restriction; CRMR, calorie-restriction meal replacement; CRF, calorie-restriction with fish oil; CRMRF, calorie-restriction meal replacement with fish oil; OGTT, oral glucose tolerance test; DEXA, dual-energy x-ray absorptiometry; BP, blood pressure; MAP, mean arterial pressure; HOMA-IR, homeostasis model assessment of insulin resistance; QUICKI, quantitative insulin sensitivity check index; EDA, eicosadienoic acid; AA, arachidonic acid; ALA, α-linolenic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; Hb, hemoglobin; CRP, C-reactive protein; IL, interleukin; TC, total cholesterol; LDLc, low-density lipoprotein-cholesterol; SD, standard deviation; ANOVA, analysis of variance; PC, postprandial glucose

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1. Introduction

Numerous countries are experiencing increasing rates of obesity, metabolic syndrome (MetS), and type 2 diabetes mellitus (T2DM) (Ford, Giles, & Dietz, 2002; Kahn, Buse, Ferrannini, & Stern, 2005), which could be attributed to genetic factors, sedentary daily lifestyle, lack of physical activity, and migration. The modified National Cholesterol Education Program, Adult Treatment Panel III (Expert Panel on Detection and Treatment of High Blood Cholesterol in Adults, 2001), and World Health Organization (1999) guidelines (WHO) define MetS as having 3 or more of the following criteria: (1) central obesity with a waist circumference (WC) ≥90 cm in men and ≥80 cm in women; (2) serum TG levels ≥150 mg/dL; (3) HDLc levels <40 mg/dL in men and <50 mg/dL in women; (4) systemic hypertension with a systolic blood pressure (SBP) ≥130 mm Hg and a diastolic blood pressure (DBP) ≥85 mm Hg; and (5) fasting plasma glucose (AC) levels >100 mg/dL. However, few studies have evaluated the dietary profiles of MetS patients. Dietary interventions are needed to reduce insulin resistance in MetS patients and to improve the various cardiometabolic factors associated with MetS.

Previous studies have established that changing macronutrient composition and intake, independently of calorie ingestion and macronutrient quality, can promote weight loss and increase insulin sensitivity. Studies have demonstrated the benefits to patients with metabolism-related disorders of restricted dietary calories (Azadbakht, Mirmiran, Esmaillzadeh, Azizi, & Azizi, 2005; Josse, Jenkins, & Kendall, 2008; Konig, Deibert, Frey, Landmann, & Berg, 2008) and increased intake of certain macronutrients (Flachs, Rossmeisl, & Kopecky, 2014; Gadgil et al., 2013; Rizza et al., 2009), particularly n-3 long-chain polyunsaturated fatty acids (LC-PUFA). de Mello et al. (2011) reported that MetS patients who consumed a healthy diet that included fatty fish and wholegrain products for 12 weeks had reduced levels of markers of inflammation and endothelial dysfunction, such as plasma E-selectin and high-sensitivity C-reactive protein (hsCRP) (de Mello et al., 2011; Su, Lee, Cheng, & Huang, 2014). A prospective epidemiological study further indicated that fish intake may prevent the development of insulin resistance (Feskens et al., 1995).

Some studies have identified the development of cardiovascular disease and certain cancers with a higher dietary intake ratio of n-6 to n-3 PUFAs (Halberg, Cornelissen, & Singh, 2011; Isomaa et al., 2001; Vaughan, Hassing, & Lewandowski, 2013). In several countries, diets tend to be high calorie, with inadequate lipid profiles, high levels of saturated and n-6 PUFAs, and less than optimal levels of n-3 PUFAs. Indeed, n-3 PUFAs have been reported to compete with n-6 PUFAs as substrates for cyclooxygenases and n-3 PUFAs often exert the inverse or reverse physiological effects of n-6 PUFA eicosanoid products (Horrobin, 1993). Therefore, the blood n-6/n-3 ratio may represent a useful clinical biomarker by which to predict chronic disease (Monteiro et al., 2014).

Clinical studies of n-3 LC-PUFA supplementation in patients with T2DM (Gadgil et al., 2013; Rizza et al., 2009; Woodman et al., 2002) have shown inconsistent results in terms of improvements in insulin sensitivity. However, some studies have found that n-3 LC-PUFA supplementation may increase insulin sensitivity in patients with impaired glucose tolerance (Rizza et al., 2009) and in T2DM patients (Woodman et al., 2002). Therefore, the purpose of our study was to determine the effects of calorie-restriction diets, with or without n-3 LC-PUFA supplementation and meal replacement, on metabolic responses and circulating fatty acid profiles in obese MetS patients.

2. Materials and methods

2.1. *Participants*

This study was conducted in Taipei, Taiwan at Taipei Medical University (TMU), Taipei Medical University Hospital (TMUH), and Wan Fang Hospital (WFH). Volunteers were recruited in Xinyi County, Taipei, Taiwan between May 2012 and March 2013, and reviewed for inclusion in a 12-week trial (Fig. 1). The inclusion criteria were based on the diagnostic criteria for MetS described in the modified National Cholesterol Education Program, Adult Treatment Panel III (Expert Panel on Detection and Treatment of High Blood Cholesterol in Adults, 2001), and World Health Organization (1999) guidelines: (1) age ≥20 years, (2) body mass index (BMI) ≥24 and ≤35 kg/m^2, and (3) waist circumference (WC) ≥90 cm in men or ≥80 cm in women. Participants were also required to have at least two of the following conditions: (1) triacylglycerol (TG) levels ≥150 mg/dL, (2) high-density lipoprotein-cholesterol (HDLc) levels <40 mg/dL in men or <50 mg/dL in women, (3) fasting blood glucose (FBG) levels >100 mg/dL, or (4) systemic hypertension with systolic blood pressure (SBP) ≥130 mm Hg and diastolic blood pressure (DBP) ≥85 mm Hg (Alberti et al., 2009; Piatti et al., 1995).

Participants with a history of cardiovascular events, acquired immunodeficiency syndrome (AIDS), cancer or another neoplastic condition, alcohol or substance abuse, or cardiovascular, hepatic, renal, metabolic, endocrine, psychiatric, cerebrovascular or peripheral vascular disease were excluded from analyses. Participants who were pregnant, had a history of hypersensitivity to fish products, were taking an n-3 PUFA dietary supplement, or were taking any type of lipid-lowering, antihypertension, or hypoglycemic medication were also excluded.

2.2. *Study design*

This single-center open-label parallel-arm controlled trial was registered at ClinicalTrials.gov (NCT01768169). The study was approved by the Ethics Committee of the Joint Institutional Review Board at TMU, and conducted in accordance with the Helsinki Declaration. Written informed consent was obtained from all study participants prior to participation. The participants were randomly assigned to one of four study groups initially: (1) calorie-restriction (CR; n = 50), (2) calorie-restriction meal replacement (CRMR; n = 47), (3) calorie-restriction with fish oil (CRF; n = 44), or (4) calorie-restriction meal replacement with fish oil (CRMRF; n = 47). The study was conducted over the course of 12 weeks, with an initial and final visit to either TMUH or WFH. During each visit, patients' medical histories were recorded and patients received a two-hour oral

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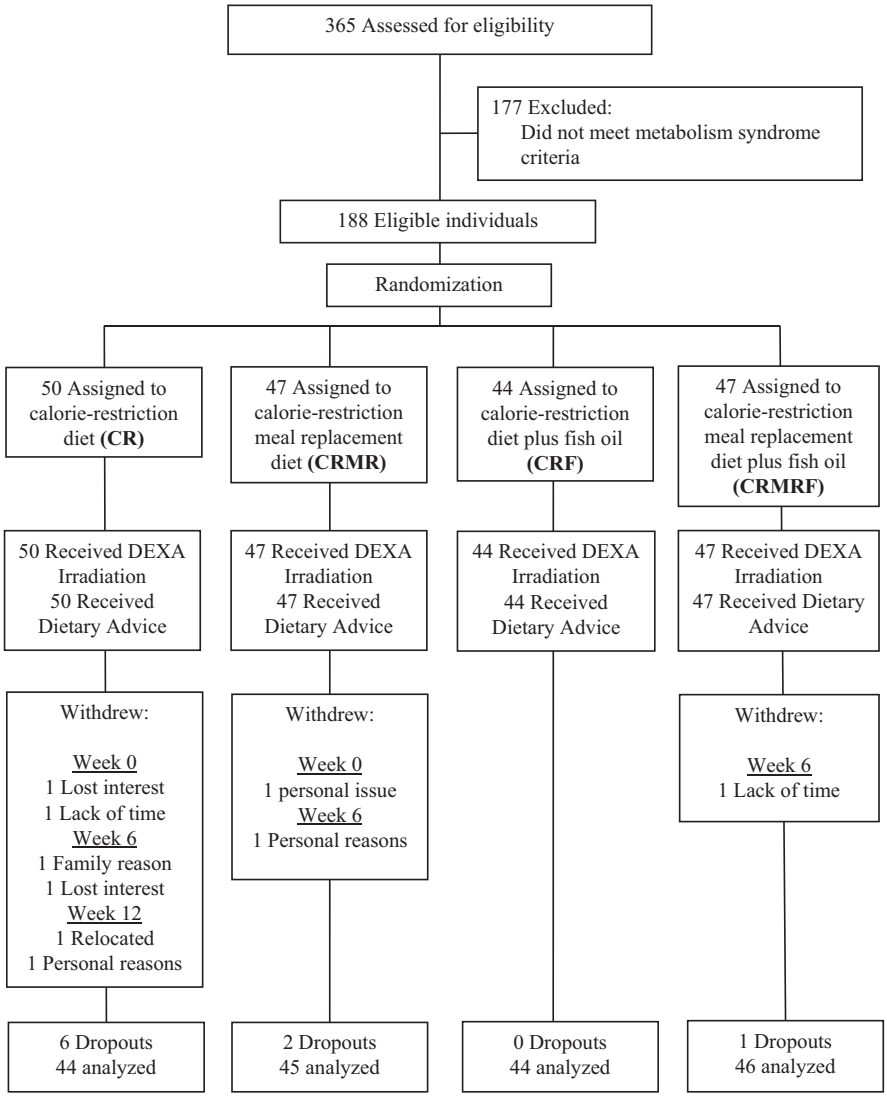

Fig. 1 – Flow-chart diagram of the participant selection process. The participants were assigned to one of the following dietary groups: calorie-restriction (CR), calorie-restriction meal replacement (CRMR), calorie-restriction with fish oil (CRF), or calorie-restriction meal replacement with fish oil (CRMRF). The 12-week trial was completed by 179 participants.

glucose tolerance test (OGTT), were measured for anthropometric variables, and had their blood drawn for biochemical analyses.

2.3. *Study diet and dietary assessment*

Based on the recommendations of the US National Institutes of Health (NHLBI Obesity Education Initiative Expert Panel on the Identification, Evaluation, and Treatment of Obesity in Adults, 1998), the CR diet provided ≥1500 kcal/day, which was 500–800 kcal/day less than the participants' regular daily dietary intake. The caloric contributions of the CR diet were 53.4% from carbohydrates, 17% from proteins, and 29.6% from lipids. The CR group received the CR diet only. The CRMR group received the CR diet and Low Calorie Nutrition Drink Mix powder (Herbalife, Los Angeles, CA, USA) for partial meal replacement, of which the total caloric contributions were 55.3% from carbohydrates, 17% from proteins (the powder provides 8 g of protein), and 27.7% from lipids. The CRF group received the CR diet and 10 Herbalifeline n-3-rich fish oil capsules containing 2.13 g of n-3 LC-PUFA for partial lipid replacement, of which the caloric contributions were 52.3% from carbohydrates, 16.5% from proteins, and 31.2% from lipids. The CRMRF group received the CR diet, the meal replacement powder, and the fish oil supplement for partial protein and lipid replacement; the overall caloric contributions for this diet were 53.8% from carbohydrates, 16.8% from proteins, and 29.4% from lipids.

The CR diet and the CRMR diet provided 10% of total calories from saturated fat. The protein sources were animal- and plant-based, and the carbohydrate sources were rice products. The participants followed the guidelines of the Department of Dietetics at TMUH during the 12-week intervention. Various calorie-controlled boxed lunches were provided to participants, and all participants were advised to substitute the meal replacement for dinner and to prepare their own breakfasts. Sample menus and information on the calorie content of

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various breakfasts were provided to participants during meetings of a nutrition course. Participant compliance was assessed during 10 weekly meetings of a nutrition course that provided instruction on healthy diets, exercise, dietary habits, and dietary behavior modification. The course was conducted every Friday afternoon by registered dieticians, and all participants met individually with a registered dietician to address any additional dietary issues.

2.4. *Anthropometric measurements*

Participants' body weights and heights were measured, and their BMIs were calculated during each of the two visits. Participants' body compositions were measured by a technologist certified by the International Society for Clinical Densitometry, using dual-energy x-ray absorptiometry (DEXA). Each participant's total body-fat mass of the trunk, extremities, and android and gynoid regions was determined using a Lunar Prodigy dual-energy x-ray absorptiometer (GE Healthcare, Madison, WI, USA). The DEXA scan data were analyzed using Lunar enCORE 2006 software Version 10.50.086 (GE Healthcare). All measurements were performed using one scanner at WFH. No changes were made in the hardware or software during the course of the trial.

2.5. *Analysis of clinical and biochemical variables*

During each of their two visits, the medical histories and selected clinical and biochemical measurements of patients were collected. Blood pressure (BP) was measured from the right arm with participants seated after a 5-minute rest period by using an Omron HEM-7230 automatic sphygmomanometer (Kyoto, Japan). BP measurements were performed in triplicate at 5-minute intervals and were averaged to obtain the mean arterial pressure (MAP), the SBP, and the DBP (Guevara-Cruz et al., 2012). The 2-hour OGTT was conducted after an overnight fast, and the blood glucose and serum insulin levels were determined at 0, 15, 30, 45, 60, 90, and 120 min following the consumption of 75 g of glucose. The homeostasis model assessment of insulin resistance (HOMA-IR) and the quantitative insulin sensitivity check index (QUICKI) were performed as described previously (Piatti et al., 1995). The equations used to calculate the MetS Z-scores were as follows: male Z-score = [(40-HDLc)/10.3] + [(TG-150)/66.5] + [(FBG-100)/13.4] + [(waist-102)/8.5] + [(MAP-100)/10.0]; female Z-score = [(50-HDLc)/12.4] + [(TG-150)/66.5] + [(FBG-100)/13.4] + [(waist-88)/11.7] + [(MAP-100)/10.03] (Johnson et al., 2007).

Blood samples were collected and centrifuged to obtain the serum samples, which were stored at –80 °C until biochemical analysis. The fatty acid methyl ester profiles of the serum samples were analyzed using gas chromatography, using methods described previously (Chiu et al., 2003). Composition data were calculated as the weight percentages of the total fatty acids. The n-6 and n-3 PUFAs and the n-6/n-3 ratio were determined as described previously (Lin et al., 2012). The n-6 PUFAs examined included linoleic acid (LA, C18:2), eicosadienoic acid (EDA, C20:2), and arachidonic acid (AA, C20:4); the n-3 PUFAs examined included α-linolenic acid (ALA, C18:3), eicosapentaenoic acid (EPA, C20:5), docosapentaenoic acid (DPA, C22:5), and docosahexaenoic acid (DHA, C22:6).

The total protein, albumin, hemoglobin A1c (Hb_{A1c}), C-reactive protein (CRP), and interleukin-6 (IL-6) levels, liver function index (glutamate-oxalate and glutamate-pyruvate transferases), kidney function index (blood urea nitrogen and creatinine), serum TG, total cholesterol (TC), low-density lipoprotein-cholesterol (LDLc), and HDLc levels were then measured from the serum samples. Total protein, albumin, TG, and TC levels were measured using an Ortho Clinical Diagnostics VITROS 950 automated analyzer (Johnson & Johnson, New Brunswick, NJ, USA). Serum LDLc, HDLc, and CRP levels were determined using a TBA-c16000 automated analyzer (Toshiba, Tokyo, Japan). Serum glucose levels were determined using an Ortho Clinical Diagnostics VITROS 5.1 FS automated analyzer (Johnson & Johnson), according to a hexokinase method, using VITROS chemistry products and GLU slides. Serum insulin levels were determined using a radioimmunoassay kit (DIA Source, Lovain-La-Nueve, Belgium). Serum HbA1c was determined using an HLC-723 GHb G7 analyzer (Tosoh, Tokyo, Japan). Serum IL-6 levels were determined using a Human IL-6 Quantikine ELISA Kit (R&D Systems, Minneapolis, MN, USA).

2.6. *Statistical analysis*

Data are presented as the mean ± standard deviation (SD) of each variable. Differences between the baseline characteristics of the treatment groups were compared using Student's t tests. The time × diet interaction was expressed as a P value derived from a repeated-measures analysis of variance (ANOVA). Changes from the baseline to the study endpoint in the serum levels of IL-6, LA, ALA, EDA, EPA, DPA, and DHA, and changes in the serum ratios of n-6/n-3, AA/EPA, and AA/DHA were compared using a one-way ANOVA. 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 Version 9.1.3 with Service Pack 3 computer software (SAS Institute, Cary, NC, USA).

3. Results

3.1. *Participant characteristics*

Fig. 1 displays a flow-chart diagram of the participant selection process. Nine of the 188 participants withdrew for various reasons, including loss of interest (n = 2), lack of time (n = 3), unspecified personal reasons (n = 2), family-related reasons (n = 1), and work-related reasons (n = 1). The remaining 179 participants completed the 12-week trial. The groups displayed nonsignificant differences in age (50.0 ± 12.6 years) and baseline BMI (29.7 ± 4.2 kg/m^2). In addition, the participants had no significant changes in nutritional status, hepatic profiles, renal function, or routine hematological parameters throughout the course of the trial. The dietary interventions were well tolerated by all of the participants, and no adverse events occurred during the trial.

3.2. *Glycemic response and blood lipid profiles*

Data on glucose metabolism indicated consistent and significant reductions following the dietary interventions in the AC

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and postprandial glucose (PC) increments, serum insulin levels, and HOMA-IR and QUICKI scores in all groups ($P < 0.05$), with the exception of AC in the CR group ($P = 0.082$). We observed nonsignificant changes in HbA1c in all groups post-intervention. We observed significant reductions in MAP in comparison with the baseline MAP in all groups post-intervention ($P < 0.05$). We also observed significant changes in the TC levels in the CRMRF group following the interventions; however, the changes in TC in all other groups were nonsignificant. A repeated-measures ANOVA indicated a significant time × diet interaction for TC in all of the study groups. We observed nonsignificant time × diet interactions for the glucose metabolism variables and other lipid variables (Table 1).

We also evaluated the effect of gender during the treatments (Table 2). From a selected biochemical parameters viewpoint, all treatment groups showed significant improvements in both male and female subjects; however, a repeated-measures ANOVA indicated significant time × gender interactions for TG in CRMR and CRMRF groups and for HDLc in CRMRF group.

3.3. *Metabolic syndrome severity and inflammatory status*

As shown in Table 1, the MetS severity Z-scores decreased significantly in each group after the interventions ($P < 0.05$). However, all groups had nonsignificant interactions of time × diet with MetS severity Z-scores ($P = 0.447$). We observed significant reductions in the IL-6 serum levels over baseline values in the CRMR, CRMRF, and CRF groups, with a significant time × diet interaction for IL-6 ($P = 0.003$). However, serum hsCRP levels showed nonsignificant changes following treatment, with a nonsignificant time × diet interaction for hsCRP. We also observed the significant changes of selected anthropometric and clinical parameters in level of HbA1c, triacylglycerol, IL-6, android (%), gynoid (%), total body fat and MetS severity Z-score (all for $P < 0.05$) (Table 3).

3.4. *Fatty acid profiles and n-6/n-3 ratios*

Table 4 displays the changes following the dietary interventions in the AA, EDA, EPA, and DPA levels, and changes in the n-6/n-3, AA/EPA, and AA/DHA ratios. We observed a significant postintervention reduction in serum AA in the CRF group in comparison with the baseline value. Serum EPA and DHA levels increased significantly in each group after the interventions, with the exception of EPA in the CR group. Following the interventions, the n-6/n-3, AA/EPA, and AA/DHA ratios decreased significantly in the CRMR, CRF, and CRMRF groups ($P < 0.01$).

3.5. *Correlations of metabolic syndrome severity with selected variables*

Tables 5 and 6 display the correlations of changes in the MetS severity Z-scores with selected biochemical variables. The changes in the MAP, AC, and serum TG levels positively correlated with the changes in the MetS Z-scores ($r = 0.528$, $r = 0.416$, and $r = 0.606$, respectively, $P < 0.001$). The changes in the HDLc levels inversely correlated with the changes in the MetS Z-scores ($r = –0.228$, $P < 0.01$). The MetS severity Z-scores positively correlated with IL-6 levels ($r = 0.499$, $P < 0.001$) and n-6/n-3 ratios ($r = 0.489$, $P < 0.001$). Following 12 weeks of supplementation with n-3 PUFAs in fish oil, serum n-6/n-3 ratios decreased significantly ($r = –0.489$, $P < 0.001$) because of increased n-3 PUFA levels. Changes in n-3 PUFA levels inversely correlated with the changes in n-6/n-3 ratios ($r = –0.797$, $P < 0.001$), whereas changes in the n-6 PUFA levels positively correlated with changes in the n-6/n-3 ratios ($r = 0.673$, $P < 0.001$). Changes in the IL-6 levels inversely correlated with those in the n-3 PUFAs ($r = –0.335$, $P < 0.001$) and positively correlated with changes in the n-6 PUFA levels ($r = 0.370$, $P < 0.001$) (Table 5).

4. Discussion

In this single-center parallel-arm trial, we evaluated the effects of a CR diet, with or without long-term supplementation with n-3 PUFAs and/or protein-rich meal replacement, on blood pressure, glucose metabolism, insulin homeostasis, IL-6 levels, and serum lipid and LC-PUFA profiles in MetS patients. According to our research, our study is the first to identify inverse correlations between MetS severity Z-scores and changes in the n-6/n-3 PUFA ratios and IL-6 levels. Participants in all study groups experienced substantial reductions in body weight (approximately 3 to 7 kg), BMI, and WC following the interventions. The weight reductions in all groups were similar to those reported previously (Appel et al., 2005; Foster et al., 2013; Yanovski & Yanovski, 2002). During the 12-week trial, participants were highly compliant with the consumption of the fish oil supplement and/or the meal replacement, and we observed no adverse events throughout the study period.

Previous studies have established the safety of using various macronutrients as the major energy source in meal replacements (Pedersen, Jesudason, & Clifton, 2013; Rolland, Hession, Murray, Wise, & Broom, 2009), demonstrating that high-dose protein diets can reduce body weight, improve certain cardiometabolic parameters, and reduce blood pressure within 6 weeks to 3 months of regular use. We observed no severe adverse effects during the course of our study. The participants displayed no notable abnormalities in liver or kidney function or in serum biochemistry. Our anthropometric findings, such as decreased BMI and WC, indicated greater improvements in the participants receiving the CRMR, CRF, and CRMRF diets in comparison with those receiving the CR diet. Therefore, the provision of n-3 PUFAs and protein meal replacements contributed to observed improvements in MetS-related parameters in participants receiving a CR diet.

Previous studies have identified that a CR diet with supplementation with n-3 PUFA and/or protein can reduce blood glucose and TG levels (Gadgil et al., 2013; Konig et al., 2008; Tovar et al., 2012). However, the individual contributions of proteins, lipids, and reduced caloric intake to these effects have yet to be fully elucidated. Our interventions improved the pre-T2DM status of participants with high baseline AC and PC values and high HOMA-IR and QUICKI scores. The n-3 PUFAs and protein-rich meal replacements did not demonstrate synergistic effects. We observed significant reductions in TG levels in the groups treated with n-3 PUFAs, which correlated with

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Table 1 – Participants' clinical and biochemical characteristics before and after the 12-week dietary interventions.[a]

Variables	CR (n = 44)		CRMR (n = 45)		CRF (n = 44)		CRMRF (n = 46)		P[b]
	Baseline	Week 12	Baseline	Week 12	Baseline	Week 12	Baseline	Week 12	Time × diet
Anthropometry									
Body weight (kg)	77.4 ± 13.7	75.2 ± 13.9	82.5 ± 17.9	75.5 ± 17.2	77.4 ± 13.9	73.9 ± 14.1	74.9 ± 15.7	70.4 ± 15.5	0.761
BMI (kg/m^2)	30.2 ± 4.6	29.3 ± 4.7	30.3 ± 4.9	27.9 ± 4.8*	29.5 ± 3.2	28.1 ± 3.2*	29.0 ± 4.0	27.2 ± 4.2*	0.591
Waist (cm)	94.8 ± 10.1	88.3 ± 9.3*	96.4 ± 11.9	89.4 ± 13.8*	94.6 ± 9.3	86.7 ± 9.9*	93.3 ± 10.4	86.8 ± 11.1*	0.824
Body composition									
Android (%)	49.1 ± 5.3	47.8 ± 6.4	48.6 ± 6.2	45.3 ± 7.8*	49.5 ± 5.3	47.7 ± 5.3	48.9 ± 4.9	45.9 ± 5.7*	0.641
Gynoid (%)	42.5 ± 7.9	41.7 ± 8.2	42.7 ± 8.8	39.4 ± 9.5	43.4 ± 8.2	42.3 ± 7.9	42.0 ± 8.0	39.8 ± 8.0	0.746
A/G ratio	1.18 ± 0.20	1.17 ± 0.19	1.17 ± 0.20	1.18 ± 0.20	1.18 ± 0.22	1.18 ± 0.23	1.19 ± 0.18	1.18 ± 0.20	0.969
Total fat (%)	40.2 ± 6.5	39.3 ± 7.0	40.2 ± 7.3	36.9 ± 8.6	40.9 ± 6.4	39.5 ± 7.2	39.5 ± 6.6	37.0 ± 6.2	0.669
Lean mass ratio	1.56 ± 0.49	1.64 ± 0.58	1.58 ± 0.54	1.89 ± 0.83	1.50 ± 0.41	1.58 ± 0.48	1.60 ± 0.43	1.80 ± 0.56	0.443
Blood pressure									
SBP (mmHg)	141.9 ± 15.5	130.8 ± 15.8*	140.1 ± 15.5	126.0 ± 18.3*	142.9 ± 15.6	127.2 ± 13.8*	147.4 ± 15.2	129.5 ± 15.3*	0.550
DBP (mmHg)	86.6 ± 9.8	79.8 ± 9.2*	85.5 ± 11.2	77.7 ± 10.8*	87.5 ± 11.1	80.2 ± 10.9*	87.4 ± 10.7	79.2 ± 10.4*	0.982
MAP (mmHg)	105.0 ± 10.6	96.8 ± 10.1*	103.7 ± 11.8	93.8 ± 12.6*	106.0 ± 12.0	95.8 ± 11.3*	107.3 ± 11.2	96.4 ± 11.5*	0.850
Glucose metabolism									
FBG (mg/dL)	107.6 ± 11.2	100.6 ± 15.7	102.8 ± 9.0	96.5 ± 9.2*	104.1 ± 9.6	94.7 ± 8.3*	104.2 ± 13.3	95.2 ± 10.1*	0.809
PC (mg/dL)	148.9 ± 55.2	120.9 ± 45.1*	144.8 ± 44.9	120.8 ± 33.3*	145.6 ± 44.9	103.7 ± 31.7*	147.0 ± 59.0	118.3 ± 38.9*	0.407
Insulin (μIU/mL)	17.1 ± 7.4	11.5 ± 5.5*	17.9 ± 8.7	12.5 ± 6.2*	14.4 ± 6.0	11.6 ± 4.9*	16.3 ± 10.5	11.5 ± 6.7*	0.635
HbA1c (%)	6.1 ± 0.6	6.0 ± 0.6	5.8 ± 0.6	5.6 ± 0.5	6.0 ± 0.4	6.0 ± 0.4	5.8 ± 0.8	5.8 ± 0.7	0.679
HOMA-IR	4.37 ± 2.05	2.87 ± 1.45*	4.39 ± 2.32	3.03 ± 1.66*	3.55 ± 1.50	2.72 ± 1.26*	4.28 ± 2.84	2.71 ± 1.58*	0.846
QUICKI	0.31 ± 0.02	0.33 ± 0.02*	0.31 ± 0.02	0.33 ± 0.03*	0.32 ± 0.02	0.33 ± 0.02*	0.32 ± 0.02	0.34 ± 0.02*	0.806
Blood lipid									
TG (mg/dL)	152.3 ± 90.4	145.3 ± 97.6	177.4 ± 74.8	131.2 ± 109.8*	158.0 ± 66.6	112.4 ± 34.5*	171.9 ± 122.8	116.7 ± 48.6*	0.241
TC (mg/dL)	195.6 ± 27.9	196.2 ± 32.6	201.0 ± 37.9	193.0 ± 34.9	197.8 ± 38.0	209.1 ± 39.7	212 ± 45.1	188.5 ± 39.0*	0.012
HDLc (mg/dL)	47.0 ± 7.6	48.3 ± 8.7	46.6 ± 7.9	46.7 ± 9.6	48.1 ± 10.8	50.2 ± 11.5	46.3 ± 9.0	47.9 ± 10.1	0.905
LDLc (mg/dL)	122.3 ± 22.5	116.5 ± 28.7	129.9 ± 34.8	121.5 ± 29.0	130.3 ± 37.6	127.2 ± 33.2	137.0 ± 35.8	117.0 ± 31.1*	0.216
Inflammatory status									
IL-6 (pg/mL)	3.22 ± 2.18	2.77 ± 2.56	3.38 ± 1.93	2.06 ± 1.15*	4.40 ± 3.58	1.59 ± 1.20*	4.27 ± 2.56	2.08 ± 1.08*	0.003
CRP (mg/dL)	0.42 ± 0.65	0.35 ± 0.36	0.45 ± 0.42	0.31 ± 0.36	0.48 ± 0.49	0.32 ± 0.33	0.45 ± 0.46	0.28 ± 0.29*	0.857
MetS severity									
Z-score	1.5 ± 3.0	–0.6 ± 3.2*	1.3 ± 2.1	–1.6 ± 3.2*	1.2 ± 2.0	–1.9 ± 2.2*	1.6 ± 2.7	–1.7 ± 2.4*	0.447

BMI: body mass index, SBP: systolic blood pressure, DBP: diastolic blood pressure, MAP: mean arterial pressure, FBG: fasting blood glucose, PC: postprandial glucose, HOMA: homeostatic model assessment, QUICKI: quantitative insulin sensitivity check index, TG: triglyceride, TC: total cholesterol, HDLc: high-density lipoprotein cholesterol, LDLc: low-density lipoprotein cholesterol, IL-6: interleukin-6, CRP: C-reactive protein, CR: calorie-restriction diet, CRMR: calorie-restriction meal replacement diet, CRF: calorie-restriction diet with fish oil, CRMRF: calorie-restriction meal replacement diet with fish oil.

BMI = body weight (kg)/height^2 (m^2), MAP = (2SBP+DBP)/3, HOMA-IR = (FBG × insulin)/22.5, QUICKI = 1/[log (insulin) + log (FBG)], male Z-score = [(40-HDLc)/10.3] + [(TG-150)/66.5] + [(FBG-100)/13.4] + [(waist-102)/8.5] + [(MAP-100)/10.0], female Z-score = [(50-HDLc)/12.4] + [(TG-150)/66.5] + [(FBG-100)/13.4] + [(waist-88)/11.7] + [(MAP-100)/10.03].

[a] Data are presented as mean ± SD.

[b] P values according to a repeated-measures analysis of variance.

* $P < 0.05$, in comparison with the baseline values and according to a paired t test.

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Table 2 – Correlations of gender and selected clinical and biochemical characteristics in CR and CRMR groups.[a]

Variables	CR (n = 44)					CRMR (n = 45)				
	M (n = 9)		F (n = 36)		P[b] Time × Sex	M (n = 15)		F (n = 30)		P[b] Time × Sex
	Baseline	Week 12	Baseline	Week 12		Baseline	Week 12	Baseline	Week 12	
Anthropometry										
Body weight (kg)	93.3 ± 19.6	85.0 ± 18.9*	76.5 ± 13.9	70.3 ± 13.9*	0.100	87.2 ± 18.7	83.1 ± 18.9*	71.1 ± 12.6	66.3 ± 12.0*	0.410
BMI (kg/m^2)	31.5 ± 5.1	28.7 ± 5.0*	29.6 ± 4.76	27.1 ± 4.6*	0.408	30.1 ± 4.5	28.7 ± 4.7*	28.6 ± 3.9	26.7 ± 3.9*	0.088
Waist (cm)	104.9 ± 12.4	97.4 ± 13.6*	91.6 ± 8.6	85.0 ± 12.1*	0.673	102.6 ± 12.5	97.5 ± 12.0*	90.4 ± 7.8	83.4 ± 8.6*	0.170
Glucose metabolism										
FBG (mg/dL)	104.9 ± 10.9	99.7 ± 12.3*	101.7 ± 7.8	94.8 ± 6.5*	0.421	102.6 ± 14.2	95.8 ± 11.2*	104.7 ± 13.16	94.9 ± 9.9*	0.417
PC (mg/dL)	170.0 ± 51.6	138.1 ± 43.6*	130.9 ± 34.4	120.6 ± 24.8	0.101	141.7 ± 76.2	117.8 ± 60.3	148.6 ± 54.0	118.4 ± 30.6*	0.604
Insulin (μIU/mL)	18.2 ± 8.2	14.6 ± 7.5*	17.8 ± 9.1	11.3 ± 5.2*	0.269	15.3 ± 4.9	11.4 ± 5.6	17.2 ± 11.8	11.6 ± 7.0*	0.598
HbA1c (%)	5.92 ± 0.81	5.67 ± 0.67*	5.74 ± 0.37	5.56 ± 0.37*	0.416	5.95 ± 1.30	5.79 ± 0.90	5.81 ± 0.58	5.80 ± 0.60	0.198
HOMA-IR	4.68 ± 2.49	3.69 ± 2.06*	4.23 ± 2.25	2.66 ± 1.28*	0.403	3.85 ± 1.23	2.68 ± 1.24*	4.42 ± 3.18	2.72 ± 1.69*	0.552
QUICKI	0.31 ± 0.02	0.32 ± 0.03*	0.32 ± 0.02	0.34 ± 0.03*	0.376	0.32 ± 0.02	0.33 ± 0.02*	0.32 ± 0.02	0.34 ± 0.02*	0.921
Blood lipid										
TG (mg/dL)	210.8 ± 104.4	179.6 ± 167.7	158.9 ± 44.4	104.6 ± 42.6*	0.473	275.0 ± 200.1	142.3 ± 53.3*	139.5 ± 60.6	108.6 ± 44.9*	0.002
TC (mg/dL)	199.9 ± 44.4	195.5 ± 47.3	201.6 ± 34.6	191.7 ± 26.6*	0.397	212.3 ± 49.6	184.0 ± 36.9*	211.9 ± 44.3	189.9 ± 40.0*	0.555
HDLc (mg/dL)	43.0 ± 7.9	44.5 ± 9.9	48.5 ± 7.3	48.0 ± 9.4	0.363	39.2 ± 5.6	40.9 ± 3.6	48.5 ± 8.8	50.2 ± 10.5	0.976
Variables	CRF (n = 44)					CRMRF (n = 46)				
	M (n = 9)		F (n = 35)		P[b] Time × Sex	M (n = 10)		F (n = 36)		P[b] Time × Sex
	Baseline	Week 12	Baseline	Week 12		Baseline	Week 12	Baseline	Week 12	
Anthropometry										
Body weight (kg)	81.3 ± 17.8	77.7 ± 17.7*	75.6 ± 11.5	72.1 ± 12.1*	0.801	77.6 ± 14.9	75.6 ± 14.8*	77.0 ± 11.9	74.6 ± 12.6*	0.467
BMI (kg/m^2)	30.2 ± 3.9	28.9 ± 4.0*	29.2 ± 2.9	27.8 ± 2.8*	0.985	30.6 ± 5.3	29.8 ± 5.2*	29.5 ± 3.4	28.6 ± 3.7*	0.534
Waist (cm)	96.9 ± 12.4	89.8 ± 12.8*	93.6 ± 7.5	83.8 ± 7.8*	0.081	96.4 ± 10.6	89.5 ± 9.8*	92.3 ± 8.9	86.5 ± 8.5*	0.456
Glucose metabolism										
FBG (mg/dL)	104.4 ± 11.6	96.3 ± 12.0*	103.9 ± 8.7	94.0 ± 5.9*	0.476	109.4 ± 21.3	103.6 ± 16.3	104.8 ± 21.4	96.0 ± 13.1*	0.537
PC (mg/dL)	142.4 ± 50.7	111.2 ± 42.5	147.1 ± 42.8	100.1 ± 25.3	0.292	155.0 ± 48.0	128.8 ± 47.3*	139.1 ± 65.5	108.4 ± 39.6*	0.729
Insulin (μIU/mL)	16.3 ± 6.8	14.3 ± 6.5	13.5 ± 5.6	10.3 ± 3.3*	0.558	16.9 ± 6.1	12.5 ± 6.6*	17.3 ± 9.2	9.8 ± 2.2*	0.126
HbA1c (%)	6.02 ± 0.42	5.91 ± 0.40	6.05 ± 0.40	5.99 ± 0.35	0.553	6.22 ± 0.52	6.17 ± 0.58	5.79 ± 0.57	5.84 ± 0.51	0.300
HOMA-IR	4.00 ± 1.71	3.44 ± 1.72	3.34 ± 1.38	2.39 ± 0.80*	0.521	4.48 ± 1.97	3.21 ± 1.71*	4.19 ± 2.23	2.34 ± 0.62*	0.257
QUICKI	0.32 ± 0.02	0.32 ± 0.02	0.32 ± 0.02	0.34 ± 0.02*	0.287	0.31 ± 0.02	0.33 ± 0.02*	0.32 ± 0.02	0.34 ± 0.01*	0.221
Blood lipid										
TG (mg/dL)	194.2 ± 68.0	137.6 ± 36.1*	141.0 ± 59.7	100.6 ± 27.1*	0.251	171.1 ± 108.0	177.6 ± 112.7	122.7 ± 38.7	94.1 ± 20.2*	0.024
TC (mg/dL)	195.3 ± 31.4	201.1 ± 34.0	199.0 ± 41.1	212.9 ± 40.8*	0.400	199.1 ± 26.0	197.6 ± 29.3	189.8 ± 30.5	193.9 ± 38.2	0.320
HDLc (mg/dL)	41.8 ± 6.0	42.1 ± 6.6	51.0 ± 11.4	54.0 ± 11.4*	0.155	46.5 ± 7.9	46.4 ± 8.2	47.9 ± 7.2	51.4 ± 8.7*	0.007

BMI: body mass index, FBG: fasting blood glucose, PC: postprandial glucose, HOMA: homeostatic model assessment, QUICKI: quantitative insulin sensitivity check index, TG: triglyceride, TC: total cholesterol, HDLc: high-density lipoprotein cholesterol, CR: calorie-restriction diet, CRMR: calorie-restriction meal replacement diet, CRF: calorie-restriction diet with fish oil, CRMRF: calorie-restriction meal replacement diet with fish oil.

BMI = body weight (kg)/height^2 (m^2), MAP = (2SBP+DBP)/3, HOMA-IR = (FBG × insulin)/22.5, QUICKI = 1/[log (insulin) + log (FBG)], male Z-score = [(40-HDLc)/10.3] + [(TG-150)/66.5] + [(FBG-100)/13.4] + [(waist-102)/8.5] + [(MAP-100)/10.0], female Z-score = [(50-HDLc)/12.4] + [(TG-150)/66.5] + [(FBG-100)/13.4] + [(waist-88)/11.7] + [(MAP-100)/10.03].

[a] Data are presented as mean ± SD.

[b] *P* values according to a repeated-measures analysis of variance.

* $P < 0.05$, in comparison with the baseline values and according to a paired *t* test.

Table 3 – Changes in selected anthropometric and clinical parameters during intervention.[a]

Variable	CR (n = 44)	CRMR (n = 45)	CRF (n = 44)	CRMRF (n = 46)	P[b]
Weight change (%)	−5.20 ± 4.78	−8.56 ± 4.49	−4.65 ± 2.34	−6.26 ± 2.78	0.095
Δ Body weight (kg)	−4.0 ± 5.8	−7.0 ± 3.9	−3.5 ± 2.0	−4.6 ± 2.2	0.052
Δ Glucose AC (mg/dL)	−7.0 ± 15.2	−6.3 ± 6.6	−9.3 ± 8.0	−9.0 ± 10.1	0.424
Δ HbA1c (%)	−0.01 ± 0.29	−0.21 ± 0.25	−0.08 ± 0.21	−0.05 ± 0.31	0.004
Δ Insulin (uIU/mL)	−6.0 ± 6.3	−5.5 ± 8.6	−2.8 ± 7.0	−5.2 ± 8.8	0.286
Δ Triglyceride (mg/dL)	−7.1 ± 50.8	−46.1 ± 102.0	−45.6 ± 43.2	−55.2 ± 100.2	0.023
Δ CRP (mg/dL)	−0.07 ± 0.57	−0.17 ± 0.28	−0.48 ± 0.66	−0.09 ± 0.28	0.122
Δ IL-6 (pg/mL)	−0.46 ± 2.01	−1.32 ± 1.76	−2.81 ± 3.53	−2.18 ± 2.60	0.000
Δ Android (%)	−1.3 ± 2.9	−3.3 ± 3.6	−1.8 ± 2.0	−2.9 ± 2.4	0.002
Δ Gynoid (%)	−0.8 ± 2.0	−3.3 ± 3.0	−1.1 ± 2.8	−2.2 ± 2.2	0.000
Δ Total body fat (%)	−0.9 ± 1.9	−3.3 ± 2.7	−1.5 ± 1.7	−2.5 ± 2.3	0.000
Δ Z-score	−2.2 ± 1.8	−2.8 ± 2.2	−3.4 ± 1.7	−3.4 ± 2.1	0.013

CR: calorie-restriction diet, CRMR: calorie-restriction meal replacement diet, CRF: calorie-restriction diet with fish oil, CRMRF: calorie-restriction meal replacement diet with fish oil, IL-6: interleukin-6, CRP: C-reactive protein, male Z-score = [(40-HDLc)/10.3] + [(TG-150)/66.5] + [(FBG-100)/13.4] + [(waist-102)/8.5] + [(MAP-100)/10.0], female Z-score = [(50-HDLc)/12.4] + [(TG-150)/66.5] + [(FBG-100)/13.4] + [(waist-88)/11.7] + [(MAP-100)/10.03].

[a] Data are presented as mean ± SD.

[b] *P* values according to a repeated-measures analysis of variance.

Table 4 – Changes in serum fatty acids in each treatment group.[a]

Variables	CR (n = 44)	CRMR (n = 45)	CRF (n = 44)	CRMRF (n = 46)	P[b]
AA (%)					
Baseline	9.60 ± 1.54	9.83 ± 1.87	8.84 ± 2.37	9.04 ± 2.43	
Week 12	9.94 ± 3.51	9.85 ± 2.24	5.98 ± 1.65*	8.89 ± 1.42	
Change	0.34 ± 3.49	0.02 ± 2.52	−2.85 ± 3.23	−0.15 ± 3.15	<0.001
ALA (%)					
Baseline	23.9 ± 3.7	25.0 ± 4.1	24.5 ± 3.4	24.7 ± 4.6	
Week 12	23.5 ± 3.0	25.5 ± 4.7	24.8 ± 2.9	23.9 ± 2.9	
Change	−0.42 ± 4.38	0.42 ± 5.93	0.27 ± 4.19	−0.79 ± 4.48	0.589
EDA (%)					
Baseline	3.04 ± 1.93	2.03 ± 2.44	2.75 ± 3.04	2.90 ± 4.06	
Week 12	6.32 ± 2.41	1.93 ± 4.72	2.56 ± 1.42	0.99 ± 0.91	
Change	3.29 ± 3.03	−0.10 ± 5.17	−0.19 ± 3.47	−1.91 ± 4.09	<0.001
EPA (%)					
Baseline	1.78 ± 0.59	1.61 ± 0.57	1.55 ± 0.90	1.39 ± 0.63	
Week 12	1.87 ± 0.45	2.67 ± 0.76*	3.36 ± 1.87*	4.37 ± 1.85*	
Change	0.09 ± 0.77	1.07 ± 0.95	1.81 ± 2.00	2.98 ± 2.02	<0.001
DPA (%)					
Baseline	1.94 ± 1.73	1.54 ± 2.32	1.51 ± 1.85	1.39 ± 1.57	
Week 12	1.37 ± 1.76	1.97 ± 3.13	0.93 ± 0.99	2.81 ± 2.10*	
Change	−0.57 ± 2.62	0.43 ± 3.99	−0.58 ± 2.31	1.42 ± 2.90	0.005
DHA (%)					
Baseline	3.19 ± 1.83	3.39 ± 1.05	3.30 ± 1.71	3.39 ± 1.95	
Week 12	4.26 ± 1.91*	5.80 ± 2.99*	5.56 ± 2.41*	5.95 ± 1.89*	
Change	1.06 ± 2.74	2.41 ± 3.14	2.26 ± 3.11	2.55 ± 2.72	0.069
n-6/n-3					
Baseline	0.95 ± 0.18	0.91 ± 0.17	0.89 ± 0.14	1.00 ± 0.34	
Week 12	0.94 ± 0.16	0.77 ± 0.21*	0.70 ± 0.12*	0.70 ± 0.14*	
Change	0.03 ± 0.27	−0.11 ± 0.28	−0.21 ± 0.19	−0.28 ± 0.35	<0.001
AA/EPA					
Baseline	6.11 ± 2.56	7.32 ± 4.50	7.10 ± 3.44	7.79 ± 5.67	
Week 12	5.55 ± 2.32	3.81 ± 0.89*	2.47 ± 1.61*	2.37 ± 1.02*	
Change	−0.56 ± 3.35	−3.51 ± 4.66	−4.88 ± 4.20	−5.87 ± 6.18	<0.001
AA/DHA					
Baseline	3.70 ± 1.67	3.33 ± 1.84	3.10 ± 1.63	3.42 ± 2.13	
Week 12	3.01 ± 2.63	2.10 ± 0.98*	1.19 ± 0.45*	1.65 ± 0.57*	
Change	−0.69 ± 2.94	−1.24 ± 1.95	−2.19 ± 2.02	−2.18 ± 2.75	0.008

CR: calorie-restriction diet, CRMR: calorie-restriction meal replacement diet, CRF: calorie-restriction diet with fish oil, CRMRF: calorie-restriction meal replacement diet with fish oil, AA: arachidonic acid (C20:4 n-6), ALA: α-linolenic acid (C18:3 n-3), EDA: eicosadienoic acid (C20:2 n-6), EPA: eicosapentaenoic acid (C20:5 n-3), DPA: docosapentaenoic acid (C22:5 n-3), DHA: docosahexaenoic acid (C22:6 n-3).

[a] Data are presented as mean ± SD.

[b] *P* values according to a one-way analysis of variance.

* $P < 0.05$, in comparison with the baseline values and according to a paired *t* test.

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Table 5 – Correlations among changes in metabolic syndrome-related parameters.

Variables	Correlation coefficient (r)					
	ΔWaist (cm)	ΔMAP (mmHg)	ΔFBG (mg/dL)	ΔTG (mg/dL)	ΔHDLc (mg/dL)	ΔZ-score
Δ Waist (cm)	1	–0.009	0.035	–0.032	0.006	0.272**
Δ MAP (mmHg)		1	0.075	–0.014	0.188*	0.528**
Δ FBG (mg/dL)			1	–0.038	0.003	0.416**
Δ TG (mg/dL)				1	–0.035	0.606**
Δ HDLc (mg/dL)					1	–0.228*
Δ Z-score						1

*P < 0.01, **P < 0.001.
MAP: mean arterial pressure, FBG: fasting blood glucose, TG: triglyceride, HDLc: high-density lipoprotein cholesterol.

the MetS severity Z-scores. However, the ability of supplementation with n-3 PUFA alone to reduce the severity of cardiometabolic symptoms in MetS patients requires further investigation.

We also observed significant interactions of time × gender for decreasing triglyceride and increasing HDLc level in female subjects under CRMRF diet. Recent studies of n-3 PUFA intervention showed consistent dose-dependent effect for decreased triglycerides (Argo et al., 2015; Oh et al., 2014). Furthermore, the significantly higher EPA incorporation rate and level in selected tissues was observed in female (Walker et al., 2014), therefore the impact of sex hormone level should be considered (Decsi & Kennedy, 2011). Besides, gender differences were observed in anthropometric and clinical parameters under calorie-restricted condition (Ahmed et al., 2009; Wong et al., 2012). Thus, more studies are needed to elucidate the aforementioned interaction between gender and diets.

The majority of our study participants (95%) demonstrated a pre-T2DM status at baseline, with elevated blood glucose levels and a high BMI. These parameters showed improvement following treatment with the various CR diets, as indicated by increased insulin sensitivity in the 2-hour OGTT. However, the effects of the macronutrient components of the various treatments on insulin sensitivity were nonsignificant. This result indicated that improvements in MetS severity in obese participants are more likely to be attributable to the low-calorie condition than to the macronutrients in the dietary treatments. After the interventions, we observed alleviated MetS symptoms in >38% of the participants in the CR (17/44), CRMR (28/45), CRF (29/44), and CRMRF (28/46) groups, with the greatest improvements in blood glucose and hypertension levels. These results indicate that blood glucose levels and blood pressure could potentially be used as predictors of recovery from MetS.

Several studies have indicated an association between the consumption of n-3 PUFAs and the prevention of chronic diseases, including MetS (Hibbeln, Nieminen, Blasbalg, Riggs, & Lands, 2006; Nigam, Frasure-Smith, Lesperance, & Julien, 2009). It is believed that an abnormal cardiometabolic state in MetS patients results in chronic inflammation. In our study, the participants receiving the fish oil supplement and/or protein-rich meal replacement showed significant improvements in both serum n-6/n-3 ratios and IL-6 levels. Although we observed a decreasing n-6/n-3 ratio in the groups receiving a protein-rich meal replacement diet, the levels of decline were less substantial than in the n-3 fatty acid-supplemented groups. The effects of macronutrients (i.e., n-3 fatty acids) in certain physiological statuses (chronic disease, heavy exercise, and aging) should also be considered (McDonald, Bauer, & Capra, 2013). We further observed that the participants' MetS severity Z-scores positively correlated with the serum n-6/n-3 ratios ($r = 0.489$, $P < 0.001$) and IL-6 levels ($r = 0.499$, $P < 0.001$). In previous studies, increased dietary intake of n-3 PUFAs altered the fatty acid composition of membrane phospholipids; reduced the production of proinflammatory eicosanoids; and reduced the serum n-6/n-3 PUFA ratio (Calder, 2009; Su, Huang, Chiu, & Shen, 2003; Su et al., 2010). Increased intake of n-3 PUFAs, particularly EPA and DHA, also contributed to the production

Table 6 – Correlations among changes in inflammatory factors, LC-PUFAs, and MetS severity Z-scores.

Variables	Correlation coefficient (r)							
	ΔIL-6 (pg/mL)	ΔCRP (mg/dL)	Δ Z-score	Δ n-3 FA (%)	Δ n-6 FA (%)	Δn-6:n-3	ΔAA:EPA	ΔAA:DHA
Δ IL-6 (pg/mL)	1	0.330**	0.499**	–0.335**	0.370**	0.489**	0.353**	0.276**
Δ CRP (mg/dL)		1	0.207*	–0.025	–0.028	0.009	–0.046	0.093
Δ Z-score			1	–0.051	0.101	0.098	0.078	0.085
Δ n-3 FA (%)				1	–0.229*	–0.797**	–0.324**	–0.373**
Δ n-6 FA (%)					1	0.673**	0.384**	0.332**
Δ n-6/n-3						1	0.399**	0.438**
Δ AA/EPA							1	0.307**
Δ AA/DHA								1

*P < 0.01, **P < 0.001.
IL-6: interleukin-6, CRP: C-reactive protein, LC-PUFA: long-chain polyunsaturated fatty acids, AA: arachidonic acid (C20:4 n-6), EPA: eicosapentaenoic acid (C20:5 n-3), DHA: docosahexaenoic acid (C22:6 n-3), MetS: metabolic syndrome.

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of certain eicosanoids that display less inflammatory potential than analogs of AA. Reductions in AA metabolism, or increases in n-3 PUFA metabolites, are considered to result from classic anti-inflammatory mechanisms. However, such outcomes as reduced serum IL-6 levels and n-6/n-3 PUFA ratios may provide more meaningful indicators of the chronic inflammatory status of MetS patients than do metabolites of AA or n-3 PUFAs (de Mello et al., 2011; Hartwich et al., 2009; Nigam et al., 2009; Novgorodtseva et al., 2011).

4.1. *Limitations*

This study had a few 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, outcomes from self-administered calorie restricted dietary treatments can be inconsistent. The percentage reductions in our participants' body fat ranged from 3.5 to 7.0% (Table 1). The participants' food-selecting behaviors could represent a potential confounding factor in this study; however, the well-designed self-monitoring program and regular group meetings provided assistance to participants who encountered difficulties understanding the available food supply. Fourth, our study focused on the effects of LC-PUFA supplementation and protein-rich meal replacements 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. Larger double-blind randomized studies on MetS patients using appropriate methodologies are required to confirm our findings.

5. Conclusions

Overall, our study findings indicate that, in MetS patients, LC-PUFA supplementation and protein meal replacement under calorie-restricted dietary conditions exert beneficial effects on metabolic parameters, including blood glucose levels, TG levels, and HOMA-IR and QUICKI scores. These treatments also improve circulating fatty acid profiles by increasing the levels of n-3 PUFAs and reducing the serum n-6/n-3 PUFA ratios and reduce the levels of the inflammatory cytokine IL-6 in MetS patients.

Conflict of interest

Dr. Huang received consulting and lecture fees from Herbalife Taiwan Ltd. The funder played no role in determining the study design; in the collection, analysis and interpretation of data; in the writing of the report; or in the decision to submit the article for publication. No potential conflicts of interest are reported by the other authors.

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