The effect of a lifestyle intervention on metabolic health in young women

Sarah L Dunn1
Winnie Siu2
Judith Freund3,3
Stephen H Boutcher2
1Department of Kinesiology, University of La Verne, CA, USA; 2School of Medical Sciences, Faculty of Medicine, University of New South Wales, NSW, Australia; 3St Vincent’s Hospital, NSW, Australia

Abstract: With the increasing obesity rates in Western countries, an effective lifestyle intervention for fat reduction and metabolic benefits is needed. High-intensity intermittent exercise (HIIE), Mediterranean eating habits (Mediet), and fish oil (ω-3) consumption positively impact metabolic health and adiposity, although the combined effect has yet to be determined. A 12-week lifestyle intervention on adiposity, insulin resistance, and interleukin-6 (IL-6) levels of young overweight women was administered. Thirty women with a body mass index of 26.6±0.5 kg/m2, blood pressure of 114/66±1.9/1.5 mmHg, and age of 22±0.8 years were randomly assigned to either an intervention group receiving Mediet advice, daily ω-3 supplementation, and HIIE 3 days/week for 12 weeks or a control group. The group receiving Mediet advice, daily ω-3 supplementation, and HIIE experienced a significant reduction in total body fat mass (P<0.001), abdominal adiposity (P<0.05), waist circumference (P<0.001), systolic blood pressure (P<0.05), fasting plasma insulin (P<0.05), IL-6 (P<0.001), and triglycerides (P<0.05). The greatest decreases in fasting plasma insulin (P<0.05) and IL-6 (P<0.001) occurred by week 6 of the intervention. Significant improvements in eating habits (P<0.05) and aerobic fitness (P<0.001) were also found following the intervention. A multifaceted 12-week lifestyle program comprising a Mediet, ω-3 supplementation, and HIIE induced significant improvements in fat loss, aerobic fitness, and insulin and IL-6 levels, positively influencing metabolic health.

Keywords: metabolic syndrome, overweight, exercise, diet

Introduction

In the US, 23% of American adults have been diagnosed with metabolic syndrome (MetS),1,2 whereas in Australia the MetS prevalence is 29%.3 Factors causing MetS are complex but include a physically inactive lifestyle, an unhealthy diet made up of saturated fat and processed foods, and inherited influences.1 MetS is considered a significant risk factor for heart disease1,3 and type 2 diabetes (T2DM).1 Clinical markers of MetS include obesity, dyslipidemia, hypertension, hyperinsulinemia, and elevated glucose levels.3 Inflammation induced by proinflammatory cytokines (interleukin-6 [IL-6]) and an elevated fasting insulin, also known as insulin resistance (IR), have been implicated as early MetS markers,4 and IR has been found in young adults free of other metabolic abnormalities.5

Unfortunately, regular aerobic exercise (walking and jogging) has not resulted in significant reductions in MetS criteria (fat loss), and long-duration exercise programs have poor adherence rates and are unpopular among overweight adults.6 High-intensity intermittent exercise (HIIE) is an alternative exercise protocol that is short in duration, resulting in reduced subcutaneous and abdominal adiposity7 and decreased IR.8-10
Previous research has found greater reductions in fat mass and fasting insulin following HIIE compared with steady state exercise in a healthy young female population at 16 weeks in MetS patients. Thus, there is evidence to suggest that exposure to chronic HIIE produces improvements in body composition and IR, leading to possible reductions in inflammation, more specifically IL-6.

It has not been determined, however, whether the addition of diet modification to an HIIE program increases fat mass loss and metabolic health. For example, consuming a 2-year Mediterranean diet (Mediet) high in fibre, omega-3 (ω-3) polyunsaturated fatty acids, and fruits and vegetables and low in red meat and saturated and trans fats has been shown to be beneficial, more specifically to decrease IR and body weight. In addition, after controlling for weight loss, inflammation declined and MetS prevalence was reduced by half following the intervention. The ingestion of ω-3, abundant in the Mediet in the form of fish oils, also has beneficial effects on MetS criteria. Studies have found significant reductions in triglycerides, improved insulin signaling, stabilization of glucose homeostasis, and a reduction in fat mass when ω-3 supplementation is combined with exercise. Several aspects of the Mediet may be improved by increased intake of ω-3 and adherence to the Mediet. However, it has been suggested that a multi-intervention approach includes the adoption of a Mediet and regular exercise involvement.

The combined effects of HIIE, a Mediet, and ω-3 ingestion on fat mass loss and metabolic health have not been examined. Consequently, the focus of this study was to investigate the effect of the combination of HIIE, a Mediet, and ω-3 ingestion on fat mass loss, IR, and IL-6 in overweight women.

Subjects and methods

Participants

Volunteer premenopausal and recreationally active but untrained overweight women were recruited from a university population. Thirty-two participants were randomly allocated into one of two groups — fish oil, exercise, and a Mediet (FEM) or control (CON) — by picking a paper marked “FEM” or “CON” out of a hat. Age (24±1.0 years; 22±0.6 years) and body mass index (BMI) (27.6±0.8 kg/m²; 25.7±0.5 kg/m²) were similar for both groups. Approval for the study was granted by the University of New South Wales Research Ethics Committee, and all participants signed the approved informed consent prior to study commencement. Previous HIIE only studies in females and males were conducted separately in our laboratory.

Procedures

Participants, who were advised to avoid strenuous activity and caffeine for 24 hours prior to testing, came into the laboratory after a 12-hour overnight fast. All tests were completed at the same time of day to avoid diurnal variation. Participants were screened for contraindications to exercise and regularity of their menstrual cycle, and personal/familial medical history was assessed.

Blood pressure and cardiorespiratory fitness

Resting blood pressure was assessed with a Colin Jentow monitor (Model 7000; Colin Medical, Japan) for pre- and post-test systolic blood pressure (SBP) and diastolic blood pressure levels in the laboratory. On a separate day, cardiopulmonary fitness was assessed using a TrueMax 2400 Metabolic Cart (ParvoMedics Inc., USA), and heart rate (HR) was recorded during this session using a Polar S810I telemetry system (Polar, Finland). After a 3-minute warm-up at 30 watts (W) with a set pedaling frequency of 60 revolutions per minute (RPM), the initial load was set at 45 W and was increased 15 W every minute until voluntary cessation and/or pedaling frequency could not be maintained. All sessions were performed on an electronically braked Monark cycle ergometer, 839E (Monark, Sweden), using a two-way breathing valve and nose clip (Hans Rudolph, USA). Due to the strenuous nature of the exercise session, not all participants achieved the criteria for \( VO_{2\text{max}} \)

Dietary intake

All participants were asked to complete a pre- and post-24-hour diet diary recall of food consumed on 3 separate days consisting of 2 week days and 1 weekend day. The diets were analyzed using dietary analysis software (Foodworks 2007, version 5.00; Xyris Software). Women assigned to the intervention group (FEM) ate a low glycemic Mediet and ingested three 1,100 mg fish oil (ω-3) capsules per day for 12 weeks. Each capsule contained 550 mg of eicosapentaenoic acid and docosahexaenoic acid per 1,100 mg capsule (YourHealth Group, Australia). The FEM women were given an overview of the healthy eating plan and recipes, along with a Mediterranean pyramid. After the initial diet sessions, FEM women were provided with feedback from their previous 1-day diet diary (every 3 weeks), and guidance was given for ways to progress toward the recommended Mediet. Use of a Mediet score (MDS) provided adherence information on a scale of 0 (least adherent) to 9 (total adherence). Scoring was based on the median values calculated from mean scores for all women in

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the FEM group. The CON group was asked to maintain their normal dietary habits.

Body composition
Participants completed a total body dual-energy X-ray absorptiometry (DXA) scan using a Lunar Prodigy scanner (software version 7.51; GE Corporation, USA) pre- and postintervention only, as the researchers did not have access to the equipment at the 6-week time point. Therefore, body fat was also assessed at baseline, 6 weeks, and 12 weeks using bioimpedance (Tanita, Japan) in order to obtain a 6-week measurement. Total body, whole body fat, and fat-free mass were measured. Central (abdominal) adiposity through DXA was measured by a standard technique previously described and by trunk fat obtained from regional analysis of the standard Lunar software. All DXA assessments were conducted by a trained technician blinded to the randomized groups. Participants were tested in the fasted state with no liquid 2 hours prior, for standardization purposes. BMI was calculated by dividing weight by height squared (kg/m²). Waist circumference (WC) was measured according to the International Society for the Advancement of Kinanthropometry protocol between the lower costal border (last rib) and the iliac crest at the narrowest point.

Exercise sessions
Women who were randomly assigned to the FEM group (n=15) completed 20 minutes of exercise (8-second sprint, 12-second recovery) on a manual cycle ergometer, 828E (Monark, Sweden), for each session three times a week for 12 weeks. The CON group was asked to maintain their normal exercise habits throughout the duration of the study. For the FEM women, the HIIE workload was set at 80%–85% of the individual’s peak HR throughout each session, with a cadence between 100 RPM and 130 RPM, and recovery was set at the same amount of resistance but at a cadence of 30 RPM. The participants’ average HR for a given HIIE session fell within their individual peak HR percent range, and intensity was increased when their HR fell below their peak HR percent range. All sessions were supervised, and participants performed a 5-minute warm-up and cool-down on the bike prior to and following each session. A rating of perceived exertion was assessed every 5 minutes, and participants cycled to a prerecorded compact disc counting down each HIIE sprint in a 3–2–1 fashion.

Fasting blood specimens
Fasting blood (total 300 mL) was drawn at baseline and weeks 6 and 12 from an antecubital vein into ethylenediaminetetraacetic acid (EDTA) vacutainers. Whole blood lipid profiles including triglyceride, total cholesterol, high-density lipoprotein, low-density lipoprotein, and glucose concentrations were quantified by automated enzymatic methods (Cholestech LDX, USA). The remaining whole blood in EDTA tubes was spun immediately in a chilled centrifuge (Model Megafuge 1.0R, Heraeus, Germany) at 4°C and frozen at −86°C for later analysis.

MetS score
Based on the clinical International Diabetes Federation (IDF) definition for MetS, the participants were given a point for each of the variables listed by the International Diabetes Federation and scored out of a total of 5.

Specimen analysis
Insulin, IL-6, and adiponectin were measured using commercially available enzyme-linked immunosorbent assay kits. The degree of enzymatic turnover of the substrate was determined by dual wavelength absorbance measurement for insulin (DSL 10-1600, USA), adiponectin (R&D DRP300, USA), and IL-6 (R&D HS600B, USA). Although C-reactive protein has clinical relevance regarding inflammation, IL-6 has been shown on a molecular level to increase levels of C-reactive protein in the liver and circulating blood. The Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) was calculated as follows: HOMA-IR = [fasting insulin (μLU/mL) × fasting blood glucose (mmol/L)]/22.5.

Statistics
Data analysis was completed with the Statistical Package for Social Science for Windows software (SPSS 18.1; USA). Student’s t-tests were used to examine differences between the two groups at baseline and on the delta score between pre- and post-testing. Body composition measurements (percent body fat, WC) from Tanita, insulin, HOMA-IR, and IL-6 (pre, week 6, and post) were analyzed by one- and two-way repeated-measures analysis with variance, and a post hoc Bonferroni test was administered. Due to skewness, adiponectin values were log transformed for analysis. Pearson correlation analysis was used to determine associations between all variables on crude and log-transformed values. Spearman’s rank order correlation was performed on values that remained skewed after log transformation. All results are expressed as mean and standard error. A P-value of <0.05 was considered significant.
Results

Thirty-two women were recruited and 30 completed the study, with one woman unable to tolerate the ω-3 and the other lost to follow-up. No significant differences were seen at baseline between the two groups. The FEM women (n=15) had significant increases in both absolute VO_{2peak} (15.0%, P<0.001) and relative VO_{2peak} (17.9%, P<0.001) and a significant reduction in SBP (8%, P<0.005) following the intervention compared with the CON group (Table 1).

In the FEM group, total body mass (1.9 kg, 3%, P<0.001), fat mass (2.6 kg, 8.3%, P<0.001), and percent body fat (measured by bioimpedance) (F [2, 22]=7.95, P<0.05) were significantly lower (Table 1 and Figure 1) following the intervention. Abdominal adiposity (P<0.05) significantly decreased by 0.12 kg (5.0%), trunk fat by 1.2 kg (9.2%), and WC by 3.7 cm (4.7%, P<0.001) in the FEM group compared with the CON group (Table 2).

Fasting plasma insulin significantly decreased (F [1,26, 17.58] =6.72, P<0.05) in the FEM group by 34%, and a trend in fasting HOMA-IR was found (F [1,12, 15.64] =4.33, P=0.05 [36%]) in the FEM group (Figure 2 and Table 3). The reductions in insulin and IL-6 (F [2, 28]=21.09, P<0.001) levels were highly correlated with baseline values (r=0.85, P<0.001, and r=-0.83, P<0.001), and the loss in fat mass was significantly associated with the reduction in fasting insulin (r=-0.46, P<0.05). Glucose did not change significantly between groups (Table 3).

Table 1 Body composition and cardiorespiratory fitness before and after the 12-week lifestyle intervention

<table>
<thead>
<tr>
<th></th>
<th>FEM</th>
<th>CON</th>
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<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>73.3±3.1</td>
<td>71.4±3.2*</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.6±0.8</td>
<td>26.8±0.9*</td>
</tr>
<tr>
<td>VO_{2peak} (L/min)</td>
<td>2.0±0.1</td>
<td>2.3±0.1*</td>
</tr>
<tr>
<td>VO_{2peak} (mL/kg/min)</td>
<td>27.3±1.2</td>
<td>32.2±1.2*</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>117.3±2.9</td>
<td>108.5±2.2**</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>67.6±2.8</td>
<td>61.6±2.6</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>31.5±2.4</td>
<td>28.9±2.6*</td>
</tr>
<tr>
<td>by DXA</td>
<td>%</td>
<td></td>
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<tr>
<td>Percent fat mass (%)</td>
<td>43.9±1.5</td>
<td>41.2±1.8*</td>
</tr>
<tr>
<td>by DXA</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>Fat-free mass (kg)</td>
<td>39.2±1.1</td>
<td>39.7±1.1</td>
</tr>
<tr>
<td>by DXA</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>79.4±2.2</td>
<td>75.7±2.1*</td>
</tr>
</tbody>
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Notes: *P<0.01; **P<0.05. Data as mean ± standard error. Abbreviations: BMI, body mass index; CON, control group; DBP, diastolic blood pressure; DXA, dual-energy X-ray absorptiometry; FEM, intervention group receiving fish oil, exercise, and a Mediterranean diet; SBP, systolic blood pressure; VO_{2peak}, peak cardiorespiratory fitness.

Insulin, HOMA-IR, and IL-6 at week 6 had significantly (P<0.001) decreased by 33%, 34%, and 35%, whereas no significant difference from week 6 to the post-test existed (Table 3 and Figure 2). Thus, the greatest decrease in insulin, HOMA-IR, and IL-6 occurred just 6 weeks into the 12-week intervention. No significant change in adiponectin (10%) after the intervention was found.

There was a significant decrease in triglyceride (18.1%, P<0.05; Table 3) levels for FEM women compared with CON women, which was significantly correlated with baseline levels (r=-0.67, P=0.006, Table 3). All other lipid levels and the score based on the MetS IDF definition were lower following FEM but not significantly different.

Table 4 illustrates the average daily dietary patterns of the two groups. The FEM group significantly decreased their energy intake (P<0.05), meat, poultry, and egg (P<0.05) and cholesterol in the diet (P<0.05) and significantly increased their fruit and nut consumption (P<0.05). It was found that the FEM group significantly increased their MDS from 2.5±1.1 to 4.8±1.4 (P<0.001), representing adherence to the Mediet. No significant increase in ω-3 levels or a decrease in the omega-6 (ω-6) to ω-3 ratio in the FEM group was noted.
by examining diet alone. However, when the consumption of ω-3 (capsule) was added to the analysis, the ω-6:ω-3 ratio in the FEM group was significantly lower (P<0.001) at the end of the intervention compared with the CON group.

Discussion
After the intervention, the FEM women, compared with the CON women, significantly reduced their fat mass and abdominal adiposity, WC, insulin, IL-6, SBP, and triglyceride levels. The majority of change in insulin and IL-6 had occurred by week 6 of the intervention. The FEM women conformed more to a Mediet, as confirmed by an increase in MDS and a significant reduction in saturated fat. A multifaceted lifestyle program comprising a Mediet, ω-3 supplementation, and HIIE induced significant fat mass and abdominal adiposity loss after 12 weeks and produced lower insulin and IL-6 levels after 6 weeks and 12 weeks. These results support the study hypothesis by showing that this type of intervention can significantly improve MetS risk factors.

Although the women did not meet the criteria for MetS, they were overweight (BMI =28 kg/m²) at baseline, and following the intervention a reduction in fat mass of 2.6 kg (0.12 kg abdominal adiposity) and WC (3.7 cm) was observed. These results support our previous laboratory findings involving 15 weeks of HIIE where a 2.5 kg reduction in fat mass (0.15 kg in abdominal adiposity) was found. Therefore, a shorter intervention in individuals with greater disease risk offered similar benefits. An 8-week study involving older T2DM males found no change in body mass. However, abdominal adiposity was decreased by 44%,28 and longer-duration HIIE (16 weeks) has provided body composition improvements in middle-aged obese women meeting the IDF definition of MetS.7 The mechanism(s) underlying the HIIE fat reduction effect is undetermined at this time. However, visceral fat exhibits higher catecholamine-induced lipolysis through increased expression of catecholamine receptors.29 Prior research in our laboratory noted that catecholamine levels increased significantly after a bout of HIIE.30 Collectively, these results indicate that HIIE protocols conducted for 12 weeks and longer result in significant decreases in fat mass and abdominal adiposity, reducing the risk of obesity and MetS.

Although the effect of HIIE on muscle mass has not been extensively examined, studies using DEXA have found that leg and trunk muscle mass were significantly increased in females by 0.6 kg and by 1.2 kg in males after 15 weeks and 12 weeks of HIIE.31,32 The 0.6 kg increase in leg and trunk muscle mass found after HIIE in the present study confirms the ability of this type of exercise to increase muscle mass. This effect may be important for fat loss programs, as muscle mass is typically decreased after dietary restriction31 and is typically unchanged after aerobic exercise training.32

In 2011, Esposito et al33 found improvements in body composition in overweight and obese men, some of whom met the criteria for MetS following long-term consumption (with or without caloric restriction) of a Mediet. The benefits

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**Table 3 IL-6, glucose, insulin, HOMA-IR, adiponectin, and lipids before and after the 12-week lifestyle intervention**

<table>
<thead>
<tr>
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<th>FEM</th>
<th>CON</th>
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<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>IL-6 (pg/mL)</td>
<td>1.49±0.20</td>
<td>0.93±0.13*</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>4.68±1.13</td>
<td>4.70±0.08</td>
</tr>
<tr>
<td>Insulin (µU/mL)</td>
<td>18.68±2.88</td>
<td>12.32±1.53**</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>3.99±0.76</td>
<td>2.56±0.30</td>
</tr>
<tr>
<td>Adiponectin (µg/mL)</td>
<td>6.33±1.05</td>
<td>6.94±1.20</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>4.50±0.18</td>
<td>4.33±0.19</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>0.83±0.08</td>
<td>0.68±0.06**</td>
</tr>
<tr>
<td>HDL (mmol/L)</td>
<td>1.42±0.10</td>
<td>1.37±0.10</td>
</tr>
<tr>
<td>LDL (mmol/L)</td>
<td>2.70±0.15</td>
<td>2.66±0.18</td>
</tr>
<tr>
<td>TC-HDL ratio</td>
<td>3.36±0.25</td>
<td>3.35±0.24</td>
</tr>
<tr>
<td>MetS score (out of 5)</td>
<td>0.7±0.2</td>
<td>0.4±0.2</td>
</tr>
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</table>

**Notes:** *P<0.001; **P<0.05. Data as mean ± standard error.

**Abbreviations:** CON, control group; FEM, intervention group receiving fish oil, exercise, and a Mediterranean diet; HDL, high-density lipoprotein; HOMA-IR, Homeostasis Model Assessment of Insulin Resistance; IL-6, interleukin-6; LDL, low-density lipoprotein; MetS score (out of 5), the participants’ score for meeting the International Diabetes Federation metabolic syndrome definition, with one point given to each individual criterion; TC, total cholesterol.
found in the current study of young sedentary but otherwise healthy women represent the combined benefits of HIIE and a Mediet. One possible mechanism may be due to the fact that the triglyceride levels were significantly lower following FEM compared with CON, which was most likely due to the Mediet because triglyceride improvements were not found in a prior interval sprinting training study. Total kilojoules (13%), fat (5%), saturated fat (3%), and cholesterol intake (27%) decreased in the FEM group compared with the CON group (Table 4). These results have been previously demonstrated following a 2-year Mediet (calorie reduction) and physical activity intervention in an obese population of premenopausal healthy women. The average two-point increase in MDS throughout the literature is considered substantial, as an increase of MDS as little as one point was found to significantly reduce mortality rates in populations by one-fifth. More specifically, fiber (25%) and fruit and nut (41%) consumption significantly increased, while a significant decrease (22%) was found. These dietary improvements are believed to contribute to a significant increase (22%) in the MDS score, representing adherence to the Mediet, which was associated with an improved fat mass and MetS variables seen previously, along with a reduction in diabetes risk. The system incorporated in the FEM investigation (MDS medians used) may have strengthened the association, as no relation to BMI in either men or women was found in a large Greek epidemiological study. Specific alterations within the dietary components of Mediet (less meat, poultry, and egg and greater legume intake) favorably impacted metabolic variables, lipids, and body composition following the 12-week intervention. Therefore, the combination of a Mediet (verified through an MDS) and HIIE offered additional benefits that the HIIE alone could not offer in relation to body composition and fasting whole blood triglyceride levels.

Fasting insulin decreased significantly by 32% (Table 3 and Figure 2) following the intervention and was similar to the reduction (31%) demonstrated by Trapp et al. This dramatic reduction in IR appears to be a feature of HIIE, and in healthy, nondiabetic individuals the improvement in fasting insulin and IR ranged from 23% to 33%, whereas in individuals possessing T2DM two studies have reported greater insulin sensitivity improvements of 46% and 58%. The increase in insulin sensitivity may be the result of alterations in the skeletal muscle signaling pathways and glucose metabolism enhancing sensitivity to insulin, the lower inflammatory markers, or changes to adipocytokines and hormones. Increased insulin sensitivity has also been shown with the consumption of the Mediet. As previously mentioned, the increase in fiber and fat consumption along with an increase in the MDS may have contributed to the metabolic improvements currently found that were slightly better than with HIIE alone in women.

The anti-inflammatory response (33% reduction in IL-6, Figure 2) after 6 weeks and 12 weeks in the FEM group is similar to the results of a study performed in T2DM, obese, and lean men for 12 weeks involving 60 minutes of exercise daily with a 55%, 17%, and 32% reduction in IL-6. Similarly, studies assessing the short-term consumption of the Mediet have shown reductions in low-grade systemic inflammation, which may be due to an increase in

| Table 4 Dietary intake before and after the 12-week lifestyle intervention |
|----------------------|------------------|------------------|
|                      | **FEM**          | **CON**          |
|                      | **Pre**          | **Post**         | **Pre**          | **Post**         |
| Carbohydrate (%)     | 44.6±1.9         | 49.3±3.0         | 49.3±2.1         | 45.5±3.0         |
| Protein (%)          | 18.0±1.0         | 19.6±1.2         | 17.5±0.8         | 17.3±1.2         |
| Fat (%)              | 34.7±1.5         | 30.2±2.8         | 31.1±1.9         | 31.7±2.0         |
| Saturated fat (%)    | 41.5±1.5         | 38.1±2.8         | 40.2±1.9         | 41.4±1.1         |
| Monounsaturated fat (%) | 41.7±1.0         | 41.9±2.9         | 40.8±1.7         | 40.3±1.5         |
| Polyunsaturated fat (%) | 16.7±1.0         | 20.0±1.4         | 19.0±1.4         | 18.3±1.4         |
| ω-6 fatty acids (g)  | 8.0±0.6          | 7.1±1.0          | 6.8±1.1          | 7.5±0.9          |
| ω-3 fatty acids (g)  | 0.95±0.12        | 1.04±0.21        | 0.62±0.08        | 0.76±0.09        |
| ω-6:ω-3 ratio (g)    | 9.4±0.8          | 8.9±1.3          | 11.2±1.1         | 10.0±0.4         |
| ω-6:ω-3 ratio (diet) | 9.4±0.8          | 2.5±0.3**        |                  |                  |
| Cholesterol (mg)     | 264.0±33.6       | 192.1±31.3*      | 208.8±25.8       | 285.8±26.4       |
| Fiber (g)            | 19.1±1.2         | 25.4±2.4         | 18.8±2.5         | 20.1±3.5         |
| Sodium (mg)          | 2,839.2±287.3    | 2,338.8±304.2    | 2,382.3±323.1    | 2,461.1±248.0    |

Notes: *P<0.05; **P<0.001. Data as mean ± standard error.
Abbreviations: FEM, intervention group receiving fish oil, exercise, and a Mediterranean diet; CON, control group.
cardiorespiratory fitness levels increased significantly by 15% in the intervention group and the CON women showed no change (Table 1). Similar HIIE protocols (same laboratory, varying duration) resulted in a 15%–24% increase in VO2peak. More intense HIIE programs (12–24 weeks) have noted large increases in VO2max of 41% in T2DM and older cardiac rehabilitation patients. The effect of HIIE on cardiorespiratory fitness is impressive, given that the most intense component of HIIE is anaerobic exercise, and possible mechanisms may involve enhanced cardiac contractility, increased skeletal muscle buffering capacity, increased mitochondrial biogenesis, and oxidative capacity. Also, repeated bouts of HIIE have been shown to result in a progressive increase in adenosine triphosphate generation, so that by halfway through a bout of intense HIIE, the majority of adenosine triphosphate was generated oxidatively. Significant oxidative adaptations in the exercising muscle underlie the significant increases in maximal oxygen uptake documented previously after regular HIIE.

The calculated MetS score based on the IDF definition for MetS decreased for the FEM women and increased for the CON women, although not significantly. Despite no significant reductions in the calculated MetS score within the FEM women, the improvements found in body composition, inflammation, and cardiorespiratory fitness are related to a lower risk for disease.

A limitation of this study may involve postintervention testing and menstrual cycle. All post-testing sessions were conducted in the days immediately following, with a minimum of 24 hours between the last session and the first post-test. Due to variations in the menstrual cycle between participants, not all women were measured during the same phase of the menstrual cycle (pre and post), and some variability in water weight gain with cycle phase should be considered.

Following a 12-week multicomponent intervention, significant reductions in body composition, IR, inflammation, and triglyceride levels were found. The greatest decrease in insulin, HOMA-IR, and IL-6 had occurred by week 6 of the intervention. Therefore, an intervention trial incorporating HIIE, Mediet, and ω-3 consumption improved a number of MetS criteria in young premenopausal overweight women.

**Acknowledgments**

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**Disclosure**

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