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ORIGINAL ARTICLE

Effect of a moderately hypoenergetic Mediterranean diet and exercise program on body cell mass and cardiovascular risk factors in obese women

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Objective: To assess the effects of a moderately hypoenergetic Mediterranean diet (MHMD) and exercise program on body cell mass (BCM) and cardiovascular disease risk factors in obese women.
Subjects/Methods: Forty-seven obese women, 39.7±13.2 years of age, with a body mass index (BMI) = 30.7±6.0 kg/m^2, completed the study. The following were measured at baseline, 2 and 4 months: BCM, BCM index (BCMI), body weight, BMI, fat-free mass (FFM), fat mass (FM), total body water (TBW), extracellular water (ECW) and intracellular water (ICW) using bioelectrical impedance analysis; fasting blood glucose (FBG), serum total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) and triglyceride (TG) concentrations; systolic (SBP) and diastolic (DBP) blood pressure.
Results: Body weight, BMI, FM, TC and TG significantly decreased ($P<0.001$; $P<0.002$ (TG)) at 2 and 4 months. FFM, TBW, ECW, FBG and DBP significantly decreased at 2 months ($P<0.05$ (FFM); $P<0.001$). LDL-C significantly decreased ($P<0.001$), while HDL-C significantly increased ($P<0.002$) at 4 months. BCM, BCMI, ICW and SBP remained stable over time.
Conclusion: BCM was preserved and cardiovascular disease risk factors improved in obese women placed on a MHMD and exercise program for 4 months.
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Keywords: body cell mass; body mass index; cardiovascular disease risk factors; mediterranean diet; obesity

Introduction

Obesity has become a worldwide epidemic; long-term approaches are required to decrease recidivism (Douketis *et al.*, 2005). It has been well established that obesity is related to cardiovascular disease (Lastra *et al.*, 2006). Cardiovascular disease is one of the top causes of morbidity and mortality among women, yet it is underdiagnosed, undertreated and under-researched in women (Albert 2005; Rosenfeld, 2006). Because diet and exercise play major roles in both the cause and prevention of chronic diseases (Lundberg *et al.*, 2006), health-care providers should educate women about initiating and maintaining a heart-healthy diet and exercise program as the cornerstone of primary and secondary prevention of obesity and cardiovascular disease (Albert 2005). In a meta-analysis, Douketis *et al.* (2005) reported that lifestyle and pharmacologic weight loss interventions were found to result in modest weight loss and improved cardiovascular disease risk factors; however, these alterations were reported mainly in individuals with established cardiovascular risks. Primary and secondary prevention of cardiovascular disease in women, especially obese women, is required (Rosenfeld, 2006). Specifically, there are

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few published studies in the areas of diet modifications, exercise and obesity prevention with respect to cardiovascular disease risk in women (Rosenfeld, 2006).

In addition to the lack of research in diet, obesity and cardiovascular disease risk among women, body cell mass (BCM), which includes fat-free mass (FFM) within muscle, viscera and the immune system, and may be considered the most functionally important compartment in determining energy expenditure, protein needs and the metabolic response to stress (Roubenoff, 1999), has not been evaluated in obese women placed on a weight loss program. Furthermore, no data are available on the relationship between BCM and cardiovascular disease risk factors in women. Therefore, the purpose of this study was to evaluate the effect of a moderately hypoenergetic Mediterranean diet (MHMD) and a moderately intense exercise program on the preservation of BCM and on cardiovascular disease risk factors in obese women. We also evaluated the MHMD on body weight and other body composition measures in this group of women.

Methods

Study design

This was a longitudinal study conducted in adult obese women. All participants were place on a MHMD (there was no control group). Body composition and metabolic parameters were evaluated at baseline, 2 and 4 months of the study. The study protocol was approved by the Ethics Committee of the University of Rome 'Tor Vergata', Rome, Italy. We did not use a control group because in our clinic we use this approach for weight loss, and we did not feel it was ethical to include a control group at this time. For future research, however, we plan to have a 'usual care' and compare that to a more intense version of what was presented in this paper.

Participants

Sixty women, between 25 and 70 years of age, with a body mass index (BMI) ranging from 25.0 to 47.8 kg/m^2, were recruited for the study and read and signed the informed consent form. Forty-seven women completed the study protocol and the analyses were performed only on the participants who completed all measurements.

All participants underwent a complete medical examination, which included blood pressure and heart rate assessments by the principal investigator (AA). We excluded individuals with any systemic disease, such as diabetes mellitus, liver or kidney disease, or hypertension requiring medication. We included individuals who had any cardiovascular disease risk factors, but who were not receiving any medication (for example, high serum total cholesterol (TC) concentrations, hypertension, but not yet on medications and so on).

Diet intervention

A MHMD was used. Although there are many forms of the Mediterranean diet, we used the original Nicotera Mediterranean diet. For more detailed information on this type of Mediterranean diet, refer Fidanza and Fidanza-Alberti (1963), Fidanza (1991), Ferro-Luzzi and Branca (1995), Fidanza *et al.* (2005) and De Lorgeril and Salen (2007).

Total daily energy content of the diet was determined on an individual basis, being equal to $\pm 10\%$ of the resting metabolic rate (RMR), calculated using De Lorenzo *et al.* (2001) prediction equation for the Italian population: RMR (kcal/day) for females $= 10.9 \times$ weight (kg)$-5.11 \times$ age (years)-890.

This diet was comprised of 55% carbohydrate, 25% fat, 20% protein (of which about 50% was comprised of vegetable proteins) and 30 g of fiber. No alcoholic beverages were allowed except 100 ml/day of red wine (energy content = 370 kJ/day). The Italian Recommended Dietary Allowances were incorporated to ensure proper vitamin and mineral intake (SINU, 1996). The composition of the diet in terms of foods and food combinations was planned to obtain a protein animal to vegetable ratio as close to 1:1 as possible. The weekly frequency of composition of animal foods was as follows: four for fish, two for meat and two for cheese. The daily intake of carbohydrates was derived mainly from pasta (80 g), bread (100 g) and fresh legumes (60 g). The daily intake of fruit was 500 ± 50 g and vegetables 250 ± 50 g. Vegetables were consumed raw as well as cooked, while meat and fish were grilled. Extra-virgin olive oil was consumed daily in the amount of 20 ± 5 g. Weekly dietary intake was evaluated by 24-h dietary recalls for all participants. The MHMD was evaluated by a dietetic software package (DS Medigroup, Milan, Italy). See Table 1 for the detailed components of the MHMD.

The participants received written instructions about the quantity and quality of foods, and that they were required to weigh their food daily before consumption. Each participant was provided with a list of all foods (in grams) to be consumed each week. The participants would arrive at the clinic every 2 weeks for new lists of food options to encourage variety. We also provided them with instructions on how to cook the food (grilled or boiled). Furthermore, they were strongly encouraged to add spices (for example, oregano, parsley, basil, rosemary, red pepper, onions and garlic) to increase food palatability. They were also strongly encouraged to drink 1.5–2.0 l of water a day to increase satiety. Participants purchased the foods themselves.

Physical activity assessment and intervention

Participants were asked to maintain their usual exercise habits outside of the supervised exercise program. Leisure-time and physical activity habits were monitored using a validated questionnaire (Salvini *et al.*, 2002; Salvini *et al.*, 2003). Furthermore, participants reported the daily number of hours they engaged in housework, as well as the weekly

Table 1 Breakdown of the components of the moderately hypoenergetic Mediterranean diet

	Breakfast	*Snack*	*Lunch*	*Snack*	*Dinner*
kcal	197.3 ± 26.5	83.7 ± 7.5	656.7 ± 24.9	99.7 ± 28.4	466.0 ± 15.5
MJ	0.83 ± 0.11	0.35 ± 0.03	2.75 ± 0.1	0.42 ± 0.12	1.95 ± 0.06
Total carbohydrate (g)	32.4 ± 4.2	17.8 ± 0.3	88.4 ± 11.1	25.4 ± 7.7	51.7 ± 0.4
Total protein (g)	7.9 ± 0.7	2.2 ± 0.3	36.1 ± 1.4	0.92 ± 0.1	28.3 ± 2.3
Total fat (g)	4.7 ± 1.1	0.87 ± 0.57	20.3 ± 7.3	0.24 ± 0.25	18.3 ± 1.2
Saturated fat (g)	1.7 ± 0.6	0.08 ± 0.003	4.6 ± 2.3	0.04 ± 0.02	3.1 ± 0.2
Polyunsaturated fat (g)	0.8 ± 0.4	0.6 ± 0.2	2.3 ± 0.7	0.5 ± 0.1	2.9 ± 0.3
Monounsaturated (g)	0.9 ± 0.07	0.05 ± 0.01	9 ± 0.5	0.05 ± 0.01	7 ± 0.1
Total cholesterol (mg)	12.4 ± 6.6	—	33.3 ± 27.7	—	28.3 ± 5.8
Total fiber (g)	1.4 ± 0.8	3.7 ± 1.1	11.7 ± 1.2	5.3 ± 3.5	10.7 ± 3.1

Values represent mean ± s.d.

number of hours they engaged in physical activities during leisure (walking, cycling, gardening and so on), during work (sedentary, standing, manual work, heavy manual work) and vigorous exercise (or sporting activities), including running, playing tennis, swimming and so on.

As part of the study intervention, subjects participated in a supervised exercise training (60 min of bicycling and running per day for at least 2 days per week). Participants were also seen by the principal investigator every 2 weeks at the outpatient clinic, to improve their adherence to the dietary and exercise regimens.

Body weight, height and BMI

Body weight was measured to the nearest 0.1 kg on an electronic beam scale, without clothes and shoes and after participants had voided. Height was measured to the nearest 0.5 cm using a stadiometer. Body weight and height were each measured twice per time point, and the average of the two measures was used. BMI was calculated as weight (kg)/height (m^2). Body weight was measured monthly to help motivate the participants; however, only baseline, 2- and 4-month measures were used for analyses and are presented in this paper.

Body composition analysis

Body composition was assessed using bioelectrical impedance analysis (BIA). BIA measurements were performed in the morning after an overnight fast of at least 12 h, abstinence of alcohol consumption for 48 h and absence of strenuous physical activity for 24 h before the testing day. Participants emptied their bladders within 30 min before undergoing the measurements. All measurements were performed on the dominant side, while participants lied supine on an examination table with their limbs abducted away from the trunk. Four gel electrodes were attached on defined anatomical positions on the hand, wrist, ankle and foot (Talluri *et al*., 2003; Kyle *et al*., 2004a, b). The BIA measurements were performed using an Akern BIA (Florence, Italy) and Littmann 2325VP adhesive electrodes (3M, St Paul, MN, USA). The BIA is capable of measuring: BCM, BCM index (BCMI), FFM, fat mass (FM), total body water (TBW), extracellular water (ECW) and intracellular water (ICW). All of the measures were conducted using the same bioelectrical impedance analyzer, using the same standard procedure described above, and conducted by the same experienced operator (AA).

Blood collection and analyses

Blood samples were collected after a 12-h fast. Serum TC, low-density lipoprotein cholesterol (LDL-C), serum high-density lipoprotein cholesterol (HDL-C), and triglyceride (TG) concentrations and plasma glucose concentrations were evaluated at baseline, 2 and 4 months. Serum TC and TG concentrations were analyzed by enzymatic methods (for serum TC concentrations: CHO-DPAP, Boehringer Mannheim, Mannheim, Germany; for serum TG concentrations: GPO-PAP, Roche Ltd, Basel, Switzerland). HDL-C and LDL-C concentrations were determined by direct methods (Wako Chemicals GmbH, Neuss, Germany for HDL-C; ABX Diagnostics, France for LDL-C). Plasma glucose concentration was measured by photometric determination using the glucose dehydrogenase method (Roche Ltd).

Blood pressure measurements

Sitting systolic (SBP) and diastolic (DBP) blood pressure were evaluated using a mercury Sphygmomanometer (Erkameter 300, ERKA, Bad Tölz, Germany). Three measurements were performed at intervals of 2–5 min, and the mean of the three values was considered as the blood pressure (Pickering *et al*., 2005).

Statistical analyses

We calculated that the sample size required would be 60 participants ($\alpha = 0.05$; $1-\beta = 0.90$), which would allow for a 22% attrition rate. Data analyses were performed at baseline 2 and 4 months, and were only calculated on women who completed the entire intervention. Differences for all variables were calculated using repeated measures analysis of variance. If significant main effects or interactions occurred, a Bonferroni's *post hoc* test was utilized to detect the differences. The data were analyzed using the Statistical Program for the Social Sciences (SPSS) version 10.0 for Windows statistical software (Chicago, IL, USA, 1999). Statistical significance was set *a priori* at $P = 0.05$ level of probability. All values are expressed as means ± s.d.

Results

Of the 60 participants who began the study, 47 completed the entire study, resulting in a 22% attrition rate, still meeting our sample size estimation. The 13 participants were dropped from the study due to poor diet adherence. Mean age of the participants was 39.7 ± 13.2 years.

Anthropometric and body composition results are listed in Table 2. Following the MHMD and exercise program, body weight and BMI and FM were significantly decreased ($P<0.001$) at 2 and 4 months. No significant loss of BCM was observed, nor were there any significant changes in BCMI. FFM, TBW, and ECW significantly decreased at 2 months ($P<0.01$), but not 4 months. There were no significant changes to the ICW through the course of the intervention.

Changes in lipid and glucose concentrations are listed in Table 3. Serum TC and TG levels significantly decreased at 2 and 4 months ($P<0.001$; $P<0.002$, respectively). Furthermore, LDL-C concentrations significantly decreased ($P<0.001$) at 4 months, with a consequent significant increase in serum HDL-C concentrations ($P<0.002$) at 4 months.

Blood pressure values are also listed in Table 3. SBP was not significantly changed; however, DBP significantly decreased at 2 months ($P<0.001$).

Table 2 Anthropometric and body composition changes of the women at baseline, 2 and 4 months

Parameters	*Baseline*	*2 months*	*4 months*
Participants (*n*)	47	47	47
Age (years)	39.7 ± 13.2	—	—
Body weight (kg)	80.4 ± 15.8	77.7 ± 15.5*	75.2 ± 14.7*
Height (cm)	161.9 ± 6.45	—	—
BMI (kg/m^2)	30.7 ± 6.0	29.7 ± 5.9*	28.7 ± 5.6*
BCM (kg)	30.3 ± 6.2	30.5 ± 5.8	30.8 ± 6.1
BCMI (kg/m^2)	11.5 ± 2.1	11.8 ± 2.5	12.1 ± 3.0
FFM (kg)	50.8 ± 8.9	49.2 ± 8.1**	49.0 ± 7.8
FM (kg)	29.5 ± 10.3	28.5 ± 10.4*	26.2 ± 10.0*
TBW (l)	37.6 ± 6.8	37.1 ± 6.3*	36.9 ± 6.1
ECW (l)	15.6 ± 2.5	15.2 ± 2.5*	15.4 ± 2.2
ICW (l)	21.9 ± 4.7	21.9 ± 4.5	21.6 ± 4.6

Abbreviations: BCM, body cell mass; BCMI, body cell mass index; BMI, body mass index; ECW, extracellular water; FFM, fat-free mass; FM, fat mass; ICW, intracellular water; TBW, total body water.
Values represent mean ± s.d.
*Significantly lower than baseline ($P<0.001$).
**Significantly lower than baseline ($P<0.05$).

Discussion

The purpose of this study was to evaluate the effect of a MHMD and a moderately intense exercise program on the preservation of BCM and on cardiovascular disease risk factors in obese women. We also evaluated the MHMD on body weight and other body composition measures in this group of women.

Anthropometric and body composition changes

This study had several important findings. First, the prescribed diet and exercise program resulted in weight loss that consisted of an appropriate FM loss and maintenance of BCM. In addition, we quantitatively explored losses of other related components, such as TBW, ECW and ICW. During the intervention, most of the aforementioned components were lost to varying degrees; however, none changed in a manner contradictory known to body composition relationships, particularly if the hydration of FFM is considered. FFM, TBW and ECW all significantly declined at 2 months, but remained stable through 4 months. Most importantly, both BCM and ICW were maintained, and BCM is an expression of ICW. Furthermore, BCM could lead to a more appropriate individual treatment. It has been well established that BCM is related to oxygen consumption (8–10 ml/kg/min) and

Table 3 Blood pressure and cardiovascular risk factor markers of the women at baseline, 2 and 4 months

Parameters	*Baseline*	*2 Months*	*4 Months*
Participants (*n*)	47	47	47
Serum total cholesterol concentrations (mg/dl)	207.4 ± 3.7	203.8 ± 2.9*	195.6 ± 14.9*
Low-density lipoprotein-cholesterol concentrations (mg/dl)	111.2 ± 23.5	103.5 ± 16.6	106.2 ± 20.2*
High-density lipoprotein cholesterol concentrations (mg/dl)	55.2 ± 4.2	55.4 ± 4.2	56.1 ± 9.4**
Serum triglyceride concentrations (mg/dl)	2.04 ± 0.12	2.02 ± 0.11*	1.98 ± 0.11*
Fasting plasma glucose concentrations (mg/dl)	92.5 ± 14.5	91.9 ± 13.9*	89.4 ± 11.4
Systolic blood pressure (mm Hg)	136.9 ± 13.1	135.4 ± 11.0	135.4 ± 10.4
Diastolic blood pressure (mm Hg)	84.4 ± 7.1	83.5 ± 5.9*	82.9 ± 5.8

Values represent mean ± s.d.
*Significantly lower than baseline ($P<0.001$).
**Significantly lower than baseline ($P<0.002$).

energy expenditure (2.7–3.6 kcal/h) (Kinney *et al*., 1963). Therefore, the more BCM that is preserved, the greater the likelihood of weight loss and maintaining the weight lost.

Gallagher *et al*. (2000) also reported a significant weight loss with a concomitant maintenance of FFM following a 16-week 1200 kcal/day hypoenergetic diet in postmenopausal obese. Gallagher *et al*. (2000) stated, 'Weight loss was accompanied by body composition and protein kinetic changes that appear appropriate for the magnitude of body mass change, thus failing to support the concern that diet-induced weight loss in obese postmenopausal women produces disproportionate LST (lean soft tissue) losses'. Our findings suggest that a small and appropriate fraction of weight loss consists of FFM that we can attribute to water loss; however, BCM was maintained, and the majority of weight loss was due to loss of FM.

Although the MHMD in our study was consumed under free-living conditions and nutrient intakes analyzed using food records, considerable effort was taken to ensure that this was achieved by the provision of key foods and providing very prescriptive diet information and menu plans. Despite this strong effort for diet and exercise adherence, and even though we did obtain statistically significant changes in body weight and composition, we did expect a greater weight loss based on our calculations. This discrepancy between predicted and achieved weight loss was mainly due to the fact that these were free-living adults. It is well known that individual error in the predicted values can be so high that for individual use a measured value is preferred over an estimated value (Heshka *et al*., 1993; De Lorenzo *et al*., 2001; Frankenfield *et al*., 2005); this is one of the limitations of our study. However, in the clinic, it is difficult to measure RMR, so these data are more closely related to what occurs in clinical practice. We did use a prediction formula, De Lorenzo *et al*. (2001); nonetheless, we realize that, because it is a prediction equation, errors will exist. Furthermore, during the first part of the intervention, the participants had to change the foods they consumed, weigh their foods and prepare their foods differently. These added 'burdens' may have led to participants consuming more energy than their actual energy needs. Because the participants were seen by the principal investigator every 2 weeks at the outpatient clinic, the 'burden' of the diet regimen was informally discussed with them. In general, participants stated that the regimen of the diet made them consume more energy. The participants were also strongly encouraged to drink 1.5–2 l of water a day to be well hydrated and to curtail their hunger.

Cardiovascular risk factor changes

It has been well established that weight loss will improve cardiovascular disease risk factors (Wing *et al*., 1992; Jakicic *et al*., 2001); however, there is no clear evidence on the relative effects of the combination of the MHMD and exercise on cardiovascular disease risk factors and BCM.

The improvements we found in lipid levels and DBP are similar to others (Stanko *et al*., 1992; De Luis *et al*., 2006); however, our research further adds to the literature because we also measured BCM, the most metabolically active tissue. Thus, we have shown that BCM and cardiovascular disease risk factors can improve with our diet and exercise program, both of which are important to overall health and quality of life.

There have been recently published data that have emphasized the importance the importance of α-linolenic acids in the prevention of heart disease. The Mediterranean-style diet has been the highlight of many of these 'heart-healthy' diets for the prevention of cardiovascular diseases, because it provides a diet rich in α-linolenic acids (De Lorgeril and Salen, 2006). De Lorgeril and Salen (2006) reviewed the literature on the Mediterranean diet and cardiovascular disease and reported a 50–70% reduction in the risk of cardiovascular disease after 4 years of follow-up in individuals with established coronary heart disease in the Lyon Diet Heart Study.

Salvini *et al*. (2006) conducted a randomized crossover trial whereby they compared the effects of a high-phenol extra virgin olive oil to a low-phenol extra virgin olive oil on oxidative DNA damage in 10 postmenopausal women living in Florence, Italy. Participants were asked to substitute all types of fat and oils normally consumed with the study oil (50 g/day) for 8 weeks in each period. Although this was not a weight loss study, they reported a reduction of DNA damage by high-phenol extra virgin olive oil, re-emphasizing the importance of a Mediterranean-type diet to decreasing cardiovascular disease risk factors, especially in postmenopausal women, who have a greater risk for heart disease.

Michalsen *et al*. (2006) evaluated the effectiveness of the Mediterranean diet on inflammatory markers and metabolic risk factors in patients with documented and treated coronary artery disease. They conducted a randomized controlled trial of 101 individuals, $59+8.6$ years of age (23% were women, and 80% of their participants were being treated with statins). Participants in the intervention group were placed on the Mediterranean diet ($n=48$) and were required to attend a 1-year program that provided 100 h of education on the Mediterranean diet. The control group consisted of 53 participants who were provided with written advice. Although the participants in the Mediterranean diet program increased their intakes of fish, fruits, vegetables, canola and olive oils, there were no differences between the groups with respect to C-reactive protein, fibrinogen, homocysteine, fasting insulin, TG and serum cholesterol concentrations. The researchers list several limitations to their study: (1) they had a relatively small sample size, (2) their could be other inflammatory markers that were affected by the Mediterranean diet, but they did not assess, (3) their results are not generalizable, because these were patients who were aggressively treated for coronary artery disease and (4) the control group could have made changes to their diet that may have confounded the results (Michalsen *et al*., 2006). Furthermore, although the Mediterranean diet has been shown to be cardioprotective, so has weight loss (as established in our study). The participants in Michalsen *et al*. (2006) study were overweight and did not lose weight from pre- to post-intervention.

Limitations

We realize that there are limitations to our study. First, and foremost, we did not have a control group. Second, our sample size was relatively small, although large enough to provide us with adequate statistical power. Finally, although we did our best to control dietary intake of our participants, this was difficult to do because they were free-living.

Conclusions

Our results suggest that a hypoenergetic Mediterranean diet combined with a moderately intense exercise program is effective in maintaining BCM and improving cardiovascular disease risk factors in obese women. Because BCM is metabolically active tissue, preserving this, while improving cardiovascular disease risk factors, will help individuals to further maintain metabolic rate, and be more likely to maintain their weight loss.

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