Effect of Weight Loss and Lifestyle Changes on Vascular Inflammatory Markers in Obese Women

A Randomized Trial

Katherine Esposito, MD
Alessandro Pontillo, MD
Carmen Di Palo
Giovanni Giugliano, MD
Mariangela Masella, MD
Raffaele Marfella, MD, PhD
Dario Giugliano, MD, PhD

Context Obesity is an independent risk factor for cardiovascular disease, which may be mediated by increased secretion of proinflammatory cytokines by adipose tissue.

Objective To determine the effect of a program of changes in lifestyle designed to obtain a sustained reduction of body weight on markers of systemic vascular inflammation and insulin resistance.

Design and Setting Randomized single-blind trial conducted from February 1999 to February 2002 at a university hospital in Italy.

Patients One hundred twenty premenopausal obese women (body mass index ≥30) aged 20 to 46 years without diabetes, hypertension, or hyperlipidemia.

Interventions The 60 women randomly assigned to the intervention group received detailed advice about how to achieve a reduction of weight of 10% or more through a low-energy Mediterranean-style diet and increased physical activity. The control group (n=60) was given general information about healthy food choices and exercise.

Main Outcome Measures Lipid and glucose intake; blood pressure; homeostatic model assessment of insulin sensitivity; and circulating levels of interleukin 6 (IL-6), interleukin 18 (IL-18), C-reactive protein (CRP), and adiponectin.

Results After 2 years, women in the intervention group consumed more foods rich in complex carbohydrates (9% corrected difference; $P<.001$), monounsaturated fat (2%; $P=.009$), and fiber (7 g/d; $P<.001$); had a lower ratio of omega-6 to omega-3 fatty acids (–5; $P<.001$); and had lower energy (–310 kcal/d; $P<.001$), saturated fat (–3.5%; $P=.007$), and cholesterol intake (–92 mg/d; $P<.001$) than controls. Body mass index decreased more in the intervention group than in controls (–4.2; $P<.001$), as did serum concentrations of IL-6 (–1.1 pg/mL; $P=.009$), IL-18 (–57 pg/mL; $P=.02$), and CRP (–1.6 mg/L; $P=.008$), while adiponectin levels increased significantly (2.2 µg/mL; $P=.01$). In multivariate analyses, changes in free fatty acids ($P=.008$), IL-6 ($P=.02$), and adiponectin ($P=.007$) levels were independently associated with changes in insulin sensitivity.

Conclusion In this study, a multidisciplinary program aimed to reduce body weight in obese women through lifestyle changes was associated with a reduction in markers of vascular inflammation and insulin resistance.

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THE RATE OF OBESITY AND THE numbers of dieters are increasing in parallel.[1,2] More than 54 million Americans are currently dieting, yet the epidemic of obesity continues virtually unabated with no sign of reversal.[3,4] Obesity is an independent risk factor for cardiovascular disease.[5] Adipocytes synthesize and secrete several cytokines, including tumor necrosis factor α,[6] interleukin 6 (IL-6),[7] and adiponectin,[8] the latter being one of the most abundant adipose tissue–specific adipocytokines.

Elevated levels of several proinflammatory cytokines, such as IL-6, IL-18, and tumor necrosis factor α, as well as the sensitive marker of inflammation C-reactive protein (CRP), have been found associated with proxy indicators of elevated body fat (body weight and body mass index [BMI], calculated as weight in kilograms divided by the square of height in meters) and with cardiovascular disease risk factors.[7,9-12] Moreover, several proinflammatory molecules, including CRP,[13] IL-6,[14] and IL-18,[15] have been prospectively associated with thrombotic cardiovascular events.

For the present study in obese women, we measured the serum concentrations of IL-6, IL-18, and CRP, as well as adiponectin, the novel adipocytokine with anti-inflammatory and insulin-sensitizing properties,[16] and their rela-

Author Affiliations: Center for Obesity Management, Department of Geriatrics and Metabolic Diseases (Drs Esposito and Pontillo and Ms Di Palo), Chair of Plastic and Reconstructive Surgery (Dr G. Giugliano), Department of Psychiatry (Dr Masella), and Cardiovascular Research Center (Drs Marfella and D. Giugliano), Second University of Naples, Naples, Italy. **Corresponding Author and Reprints:** Katherine Esposito, MD, Center for Obesity Management, Department of Geriatrics and Metabolic Diseases, Policlinico Universitario, Piazza L. Miraglia, 80138 Napoli, Italy (e-mail: katherine.esposito@unina2.it).

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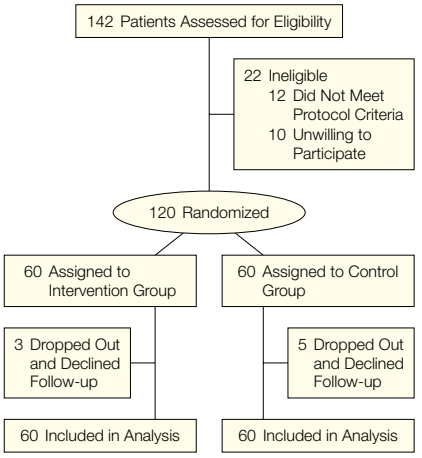
Figure 1. Flow of Patients Through the Trial



tions with anthropometric measures and insulin resistance. We then performed a randomized controlled trial of lifestyle changes designed to obtain a sustained and long-term reduction of body weight (≥10% of initial weight, maintained for 2 years) for evaluating the effect of weight loss on markers of vascular inflammation and insulin resistance.

METHODS

For this trial conducted from February 1999 to February 2002, obese premenopausal women aged 20 to 46 years were recruited from the outpatient department for weight loss of the teaching hospital at the second University of Naples, Italy. Women were sedentary (<1 h/wk of physical activity), with no evidence of participation in diet reduction programs within the last 6 months, and completed a personal health and medical history questionnaire, which served as a screening tool. Exclusion criteria were type 2 diabetes mellitus or impaired glucose tolerance (plasma glucose levels of 140-200 mg/dL [7.8-11.1 mmol/L] 2 hours after a 75-g oral glucose load), hypertension (blood pressure >140/90 mm Hg), cardiovascular disease, psychiatric problems, history of alcohol abuse (intake of ≥500 g/wk in the last year), current smoking, and any medication use. No patient was pregnant or became pregnant during the study. The study was approved by the institutional committee of ethical practice of our institution, and all study participants gave written informed consent.

Women were individually assigned to either the intervention or control group by selection of an envelope from a pile of equal numbers of envelopes for each group (FIGURE 1). The nurses who scheduled the study visits did not have access to the randomization list. However, the staff members involved in the intervention were aware of the group assignment. Laboratory staff did not know the participants' group assignments.

Women in the control group were given general oral and written information about healthy food choices and exercise at baseline and at subsequent monthly visits, but no specific individualized programs were offered to them.

Women in the intervention group were given detailed advice about how to achieve a reduction in weight of 10% or more. The program involved education on reducing dietary calories, personal goal setting, and self-monitoring (food diaries) through a series of monthly small-group sessions. Behavioral and psychological counseling was also offered. The mean caloric intake goal was set at 1300 kcal/d for the first year and 1500 kcal/d for the second year. The recommended composition of the dietary regimen was 50% to 60% carbohydrates, 15% to 20% proteins, less than 30% total fat, less than 10% saturated fat, 10% to 15% monounsaturated fat, 5% to 8% polyunsaturated fat, and 18 g of fiber per 1000 kcal. This regimen is similar to the Mediterranean-style Step I diet, which is being considered by the American Heart Association as a possible tool to lower cardiovascular risk.[17] Dietary advice was tailored to each woman on the basis of 3-day food records. These women also received individual guidance on increasing physical activity, mainly by walking, but also with swimming or aerobic ball games. Women were enrolled in the program for 24 months and had monthly sessions with the nutritionist and exercise trainer for the first year and bimonthly sessions for the second year. Compliance was assessed by attendance at the meetings and completion of the diet diaries.

Height and weight were recorded with participants wearing lightweight clothing and no shoes using a Seca 200 scale with attached stadiometer (Seca, Hamburg, Germany). Waist-hip ratio (WHR) was calculated as waist circumference in centimeters divided by hip circumference in centimeters. Twenty-four-hour nutrient intakes were calculated with food-composition tables and patients' weekly diet diaries. All women were asked to complete a 3-day food intake record and to record occupational, household, and leisure-time physical activity to assess dietary adherence and exercise activity. Foods were measured using standard measuring cups and spoons and weight-approximation diagrams.

Insulin sensitivity in the fasting state was assessed with homeostasis model assessment (HOMA) and calculated with the following formula: fasting plasma glucose (mmol/L) × fasting serum insulin (μU/mL) divided by 25, as described by Matthews et al.[18] High HOMA scores denote low insulin sensitivity (insulin resistance). Assays for serum total and high-density lipoprotein cholesterol, triglyceride, and glucose levels were performed in the hospital's chemistry laboratory. Plasma free fatty acids (FFAs) were determined as previously described.[19] Plasma insulin levels were assayed by radioimmunoassay (Ares, Serono, Italy).

Serum samples for cytokine and CRP levels were stored at –80°C until assayed. Serum concentrations of IL-6 and IL-18 were determined in duplicate using a highly sensitive, quantitative sandwich enzyme assay (Quantikine HS, R&D Systems, Minneapolis, Minn). The lower limit of detection was 0.7 pg/mL for both. High-sensitivity CRP was assayed by immunonephelometry on a Behring Nephelometer 2 (Dade Behring, Marburg, Germany). Plasma adiponectin was assessed using a commercially available radioimmunoassay kit (HADP-61HK, Linco Research, St Charles, Mo). In our laboratory, the normal means (and ranges) for these values, based on 80 healthy nonobese women matched to obese women for age and metabolic characteristics, are as fol-

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lows: IL-6, 1.9 pg/mL (0.3-12.5 pg/mL); IL-18, 129 pg/mL (50-275 pg/mL); CRP, 1.2 mg/L (0.3-10 mg/L); and adiponectin, 8.7 µg/mL (1.6-15 µg/mL).

Data are presented as mean (SD) unless otherwise stated. Data were analyzed by intention to treat. We compared baseline data using a *t* test for continuous variables and a nonparametric Wilcoxon test for IL-6, IL-18, CRP, and adiponectin. We compared risk factors and nutrient intakes after 2 years using a test based on the values at the end of follow-up and a *t* test based on differences from baseline. Results of the analysis omitting patients lost to follow-up did not differ from that including their last available records; data are therefore shown for the analysis that includes all women. Spearman rank correlation coefficients were used to quantify the relations between metabolic variables and cytokine levels. The effects of weight loss on cytokine levels were tested by means of paired *t* tests on log-transformed values and a nonparametric Wilcoxon matched test. Multivariate regression analysis tested the independent association and contribution of changes in BMI, WHR, FFA, physical activity, and plasma cytokine concentrations with the dependent variable (changes in HOMA). $P<.05$ was considered significant. All analyses were conducted using SPSS version 9.0 (SPSS Inc, Chicago, Ill).

Table 1. Clinical Characteristics of Study Participants*

	Intervention Group (n = 60)	Control Group (n = 60)	*P* Value
Age, y	34.2 (4.8)	35.0 (5.1)	.70
Weight, kg	95 (9.4)	94 (9.2)	.65
Body mass index†	35.0 (2.3)	34.7 (2.4)	.75
Waist-hip ratio	0.86 (0.07)	0.87 (0.07)	.81
SBP, mm Hg	124 (8.5)	123 (7.9)	.59
DBP, mm Hg	85 (4.7)	85 (4.9)	.61
Glucose, mg/dL	106 (14)	105 (13)	.57
Insulin, µU/mL	14 (4)	14 (4)	.45
HOMA‡	3.6 (0.4)	3.7 (0.5)	.64
TC, mg/dL	197 (23)	193 (23)	.51
HDL-C, mg/dL	46 (10)	46 (10)	.88
Triglycerides, mg/dL	142 (44)	142 (53)	.81
FFA, mmol/L	581 (102)	562 (98)	.63
IL-6, pg/mL§	4.3 (1.9-9.0)	4.1 (2.0-9.0)	.39
IL-18, pg/mL§	225 (189-291)	217 (183-289)	.45
Adiponectin, µg/mL	5.6 (2.2)	5.4 (2.1)	.50
CRP, mg/L§	3.2 (1.5-8.4)	3.4 (1.4-8.3)	.37

Abbreviations: CRP, C-reactive protein; DBP, diastolic blood pressure; FFA, free fatty acids; HDL-C, high-density lipoprotein cholesterol; IL-6, interleukin 6; IL-18, interleukin 18; SBP, systolic blood pressure; TC, total cholesterol.
SI conversion factors: to convert glucose from mg/dL to mmol/L, multiply by 0.0555; insulin from µU/mL to pmol/L, multiply by 7.175; TC and HDL-C from mg/dL to mmol/L, multiply by 0.0259; and triglycerides from mg/dL to mmol/L, multiply by 0.0113.
*Data are presented as mean (SD) except where otherwise indicated.
†Body mass index is calculated as weight in kilograms divided by the square of height in meters.
‡Homeostatic model assessment (HOMA) of insulin sensitivity in the fasting state is calculated as fasting plasma glucose (in mmol/L) × fasting serum insulin (in µU/mL) divided by 25.[18]
§Data are presented as median (interquartile range).

RESULTS

One hundred twenty women were randomly assigned to the intervention (n=60) or control group (n=60) (Figure 1). Because participants were carefully screened for exclusion criteria, both groups were comparable and relatively healthy (TABLE 1). All women were premenopausal and obese, with BMI values ranging from 30 to 49. As expected for an obese female population, serum IL-6, IL-18, and CRP levels were higher than reported in nonobese women.[9-12] In contrast, adiponectin levels were significantly lower ($P=.008$) in the obese women in the present study compared with a group of nonobese women (n=80) matched for age and metabolic and clinical characteristics (whose adiponectin levels were 8.7 µg/mL).

Spearman rank correlation coefficients between serum cytokine levels and metabolic variables are shown in TABLE 2. Univariate correlations are reported because they were affected very little by adjustment for age. Serum IL-6 levels were positively associated and adiponectin levels were negatively associated with BMI, WHR, insulin, HOMA, and FFA. To investigate which variables might account for the association between circulating IL-6 or adiponectin levels and insulin resistance, multiple regression analysis was performed. The independent variables were those significantly correlated with both IL-6 and adiponectin in univariate analysis. Only BMI ($P=.03$), FFA ($P=.02$), and HOMA ($P=.04$) were independently and significantly associated with IL-6 (positively) or adiponectin (negatively).

After 2 years of follow-up, there were 3 dropouts in the intervention group and 5 in the control group, all of which occurred after 24 weeks of follow-up. Dropouts from the intervention group showed a decrease in body weight after 24 weeks of follow-up, suggesting that they were adhering to the lifestyle changes. Baseline data showed no important differences in nutrient intake between the 2 groups (TABLE 3). After 2 years, patients in the intervention group consumed a greater percentage of calories from complex carbohydrates, protein, and monounsaturated fat; had a lower ratio of omega-6 to omega-3 fatty acids; and had lower energy, saturated fat, and cholesterol intake levels than controls. The level of physical activity increased more in the intervention group (from 64 to 175 min/wk) than in the control group (from 71 to 102 min/wk [$P=.009$]).

After 2 years, both groups had a significant decrease in body weight, BMI, WHR, blood pressure, glucose, insulin and HOMA, triglycerides, and FFA, with a larger effect in the intervention group (TABLE 4). High-density lipoprotein cholesterol increased more in the intervention group. Serum concentrations of IL-6, IL-18, and CRP were significantly reduced in those the intervention group compared with controls, while adipo-

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nectin levels were significantly increased (FIGURE 2). The magnitude and significance of the weight loss–induced difference in cytokine and CRP levels were similar when a paired *t* test was performed on $\log_{10}$-transformed values or when a nonparametric Wilcoxon matched test was used.

In the intervention group, changes in cytokine and CRP concentrations were related to the reduction in BMI (for IL-6, $r=0.35$; $P=.02$; for IL-18, $r=0.29$; $P=.04$; for CRP, $r=0.41$; $P=.008$; and for adiponectin, $r=-0.31$; $P=.02$). For evaluating the independent association of changes in HOMA with changes in serum IL-6 and adiponectin levels, a multivariate analysis was performed in which HOMA was the dependent variable and BMI, WHR, physical activity, plasma FFA, and serum IL-6 and adiponectin were the independent variables. Free fatty acids (27.6% of the variance; $P=.008$), IL-6 (15% of the variance; $P=.02$), and adiponectin (22% of the variance; $P=.007$) were independent predictors of HOMA and explained approximately 65% of the variability.

COMMENT

In this study, we tested the hypothesis that a multidisciplinary approach aimed at reducing body weight by 10% or more was effective at 2 years and reduced circulating levels of inflammatory markers of future cardiovascular events. The physiological rationales underlying these hypotheses are that (1) obesity is a difficult problem, such that at most, 10% of dieters manage to keep the weight off in the long term[20]; (2) obesity has been positively associated with insulin resistance and increased serum concentrations of vascular inflammatory markers[9-10]; and (3) adipose tissue has been proposed as a factor directly modulating proinflammatory and anti-inflammatory cytokine levels.[6-8]

In the baseline cross-sectional analysis of all 120 obese women, we observed significant associations between metabolic variables and levels of inflammatory markers. Similar to previous studies,[7,9-11] we found positive correlations among body weight, BMI, WHR, and levels of CRP, IL-6, and IL-18, suggesting that the circulating levels of these cytokines may reflect, at least in part, production by adipose tissue. Fasting serum concentrations of IL-6 were positively associated and adiponectin levels were negatively associated with all of the markers of insulin resistance measured (fasting insulin levels, HOMA, and WHR). The mechanisms whereby high IL-6 or low adiponectin levels can induce insulin resistance at the cellular level are poorly understood. However,

Table 2. Correlations With IL-6, IL-18, and Adiponectin in All Obese Women (n = 120)

	IL-6	*P* Value	IL-18	*P* Value	Adiponectin	*P* Value
Weight	0.41	.008	0.37	.007	–0.44	.007
Body mass index*	0.39	.008	0.41	.008	–0.35	.007
Waist-hip ratio	0.45	.006	0.37	.006	–0.44	.007
Glucose	0.21	.04	0.10	.09	–0.15	.04
Insulin	0.19	.03	0.21	.04	–0.27	.009
HOMA*	0.41	.008	0.13	.08	–0.34	.008
TC	0.06	.13	0.07	.14	–0.02	.37
HDL-C	–0.08	.10	0.04	.24	0.09	.10
Triglycerides	0.09	.09	0.05	.19	–0.18	.05
IL-6†	NA		0.13	.08	–0.25	.02
IL-18†	0.13	.08	NA		–0.09	.10
Adiponectin	–0.25	.02	–0.09	.10	NA	
CRP†	0.52	.006	0.10	.09	–0.35	.008
FFA	0.40	.008	0.12	.08	–0.45	.007

Abbreviations: CRP, C-reactive protein; FFA, free fatty acids; HDL-C, high-density lipoprotein cholesterol; HOMA, homeostatic model assessment of insulin sensitivity; IL-6, interleukin 6; IL-18, interleukin 18; NA, data not applicable; TC, total cholesterol.
*For definitions, see Table 1 footnotes.
†Log-transformed data.

Table 3. Nutrient Indices at Entry to Study and at 2 Years*

	Intervention Group (n = 60)				Control Group (n = 60)					
Nutrient Intake	Baseline	2 Years	Mean Change	*P* Value	Baseline	2 Years	Mean Change	*P* Value	Corrected Difference (95% CI)†	*P* Value at 2 Years
Total energy, kcal/d	2070 (175)	1650 (141)	–420	<.001	2100 (185)	1190 (174)	–110	<.001	–310 (–450 to –170)	<.001
Carbohydrates, %	58 (2.0)	55 (1.7)	–3	.005	59 (2.1)	58 (2.9)	–1	.02	–2 (–3 to –1)	.009
Complex carbohydrates, %	40 (2.5)	48 (2.1)	+8	<.001	39 (2.4)	38 (2.2)	–1	.02	+9 (4.4 to 13.6)	<.001
Fiber, g/d	16 (1.8)	25 (1.6)	+9	<.001	14 (1.9)	16 (1.7)	+2	.02	+7 (3 to 11)	<.001
Protein, %	14 (1.9)	17 (1.7)	+3	.02	14 (1.7)	14 (1.5)	+0.5	.04	+2.5 (1 to 4)	.008
Fat, %	28 (3.1)	28 (2.7)	0	.40	27 (3.3)	28 (2.9)	+1	.02	–1 (–2.5 to 0.6)	.15
Saturated	12 (2.1)	8 (1.6)	–4	.01	12 (2.0)	11 (1.5)	–0.5	.05	–3.5 (–5 to –2)	.007
MUFA	10 (2.0)	13 (1.5)	+3	.01	10 (2.0)	11 (1.4)	+1	.02	+2 (0.8 to 3.3)	.01
PUFA	6 (1.0)	7 (0.8)	+1	.02	6 (1.1)	7 (0.8)	+0.5	.02	+0.5 (–0.5 to 1.5)	.17
Omega-6/omega-3 fatty acid ratio	11 (2.0)	6 (0.8)	–5	<.001	13 (2.1)	13 (1.9)	0	.41	–5 (–8 to –2)	<.001
Cholesterol, mg/d	340 (34)	235 (29)	–105	<.001	345 (37)	332 (33)	–13	.03	–92 (–131 to –51)	<.001

Abbreviations: CI, confidence interval; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.
*Data are presented as mean (SD).
†Intervention group minus control group.

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Table 4. Cardiovascular Risk Factors at Baseline and at 2 Years*

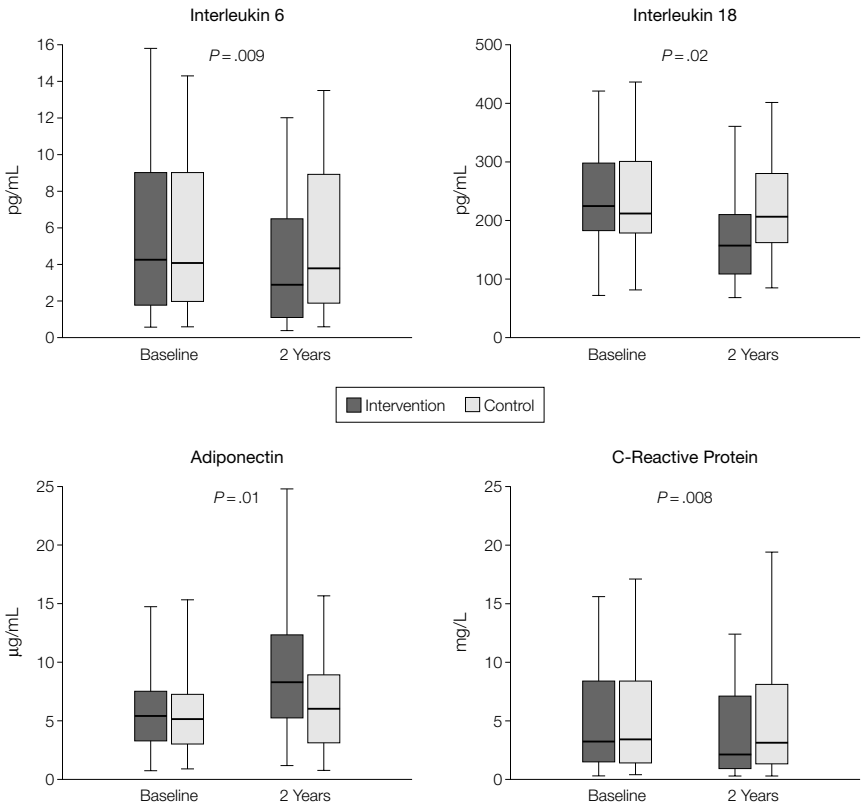
	Intervention Group (n = 60)				Control Group (n = 60)					
Risk Factors	Baseline	2 Years	Mean Change	*P* Value	Baseline	2 Years	Mean Change	*P* Value	Corrected Difference (95% CI)†	*P* Value at 2 Years
Weight, kg	95 (9.4)	81 (7.5)	−14	<.001	94 (9.2)	91 (9.0)	−3	.01	−11 (−14 to −8)	<.001
Body mass index‡	35 (2.3)	30 (2.1)	−5.2	<.001	34 (2.4)	34 (2.4)	−1	.04	−4.2 (−6.4 to −2)	<.001
Waist-hip ratio	0.86 (0.07)	0.78 (0.07)	−0.08	<.001	0.87 (0.07)	0.85 (0.07)	−0.02	.03	−0.06 (−0.09 to −0.03)	.008
SBP, mm Hg	124 (8.5)	121 (8.4)	−3	.01	124 (7.9)	122 (7.8)	−1.0	.15	−2 (−3.5 to −0.5)	.009
DBP, mm Hg	85 (4.7)	82 (4.6)	−3	.01	84.5 (4.9)	83.2 (4.5)	−1.3	.27	−1.7 (−3 to −0.4)	<.001
Glucose, mg/dL	106 (14)	97 (13)	−9	.01	105 (13)	103 (11)	−2	.16	−7 (−9 to −5)	<.001
Insulin, µU/mL	14 (4)	9 (3)	−5	.02	14 (4)	12 (3)	−2	.02	−3 (−5 to −1)	.009
HOMA‡	3.6 (0.4)	2.3 (0.3)	−1.3	.02	3.7 (0.5)	3.3 (0.4)	−0.4	.02	−0.9 (−1.3 to −0.5)	.008
TC, mg/dL	197 (62)	193 (58)	−4	.04	193 (23)	193 (23)	0	.50	−4 (−12 to 4)	.13
HDL-C, mg/dL	46 (12)	54 (12)	+8	.03	46 (12)	46 (12)	0	.40	+4 (2 to 6)	.02
Triglycerides, mg/dL	142 (44)	123 (35)	−19	.04	150 (53)	142 (44)	−8	.30	−12 (−18 to −5)	.04
FFA, mmol/L	581 (102)	419 (63)	−162	.01	562 (98)	523 (85)	−39	.11	−123 (−200 to −53)	.01
IL-6, pg/mL§	4.3 (1.9-9.0)	2.9 (1.1-6.5)	−1.4	.01	4.1 (2.0-9.0)	3.8 (2.1-8.9)	−0.3	.15	−1.1 (−1.7 to −0.6)	.009
IL-18, pg/mL§	225 (185-291)	157 (112-212)	−68	.02	217 (183-289)	206 (165-274)	−11	.24	−57 (−100 to −12)	.02
Adiponectin, µg/mL	5.6 (2.2)	8.3 (2.9)	+2.7	.02	5.4 (2.1)	5.9 (2.1)	+0.5	.13	+2.2 (1.0 to 3.5)	.01
CRP, mg/L§	3.2 (1.5-8.4)	2.1 (0.9-7.1)	−1.1	.01	3.4 (1.4-8.3)	3.1 (1.3-8.2)	−0.3	.19	−0.8 (−2.0 to −0.4)	.008

Abbreviations: CI, confidence interval; CRP, C-reactive protein; DBP, diastolic blood pressure; FFA, free fatty acids; HDL-C, high-density lipoprotein cholesterol; HOMA, homeostatic model assessment of insulin sensitivity; IL-6, interleukin 6; IL-18, interleukin 18; SBP, systolic blood pressure; TC, total cholesterol.
SI conversion factors: see Table 1.
*Data are presented as mean (SD) unless otherwise indicated; †Intervention group minus control group; ‡For definitions, see Table 1 footnotes; §Data are presented as median (interquartile range).

IL-6 increases plasma FFA and fat oxidation in humans,[21] whereas plasma adiponectin levels are positively associated with whole-body insulin sensitivity.[22] Interestingly, there is some evidence that adiponectin may participate in fatty acid and energy homeostasis.[23] It is possible that a high production of IL-6 from adipose tissue associated with low production of adiponectin may be involved in obesity-associated insulin resistance through convergent effects on increasing plasma fatty acid levels. The associations we found between FFA and IL-6 and adiponectin, both at baseline and after weight loss, seem to support this interpretation.

This study shows that weight loss can be effectively achieved in the long term by a multidisciplinary approach to lifestyle changes in obese premenopausal women. The intervention program showed improvement in the number of surrogate traditional and novel cardiovascular risk factors, which were better than those observed in controls. Our results suggest that to be successful, interventions should be multifactorial and of long duration. Otherwise, the initial efforts and enthusiasm for healthier lifestyles are eroded by external obeso-

Figure 2. Serum Concentrations of Cytokines and C-Reactive Protein at Baseline and 2 Years



Data are shown as medians, interquartile ranges, and extreme values. *P* values are for comparisons of treatment effects between the intervention and control groups.

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genic environmental forces.[24] If the lifestyles can be maintained, they will reduce the amount of weight gain and the risk of associated diseases.

The obese women we studied had yet to heed the messages about prevention of heart disease. Baseline diets in our participants contained large amounts of saturated fat, cholesterol, and refined carbohydrates and small amounts of fiber and omega-3 fatty acids. Dietary supplementation with whole grain products, legumes, fruit, vegetables, fish, and olive oil was associated with improvement of nutrient indices at 2 years (Table 3) and a substantial decrease in cardiovascular risk (Table 4) in the intervention group. Given the strict exclusion criteria, the obese women were healthy at baseline, and no cardiovascular events occurred in the 2 years of follow-up. However, diets with similar characteristics to those we used, accompanied by small weight changes, can reduce cardiac end points in high-risk patients in the first few months of follow-up.[25,26]

The vascular inflammatory markers that improved after 2 years of follow-up in the intervention group are linked to future thrombotic events through mechanisms of plaque destabilization. Consistent findings support a predictive role of CRP and IL-6 in different populations,[27] IL-18 has been identified as an independent predictor of cardiovascular death in patients with a broad spectrum of coronary artery disease,[15] and circulating levels of adiponectin are lower in patients with coronary artery disease.[28] Thus, the increased cardiovascular risk of obese persons may be seen as the result, at least in part, of increased inflammatory stimuli and decreased anti-inflammatory mechanisms.

The obesity-inflammation relationship has been addressed by previous studies[9-11,22,29,30] that were characterized by limited follow-up, absence of a control group, small numbers of patients, and lack of adiponectin data, at least for nonsurgically treated obese patients.[22] We show that a multidisciplinary program aimed to reduce body weight in obese women through lifestyle changes, including a low-energy Mediterranean-type diet and increased exercise, is feasible and gives sustained results over 2 years, as indicated by the significant reduction of markers of inflammation and improved insulin sensitivity. Although we cannot exclude that the change in physical activity and food intake may have contributed to the effects of weight loss, the potential benefits of the program justify its evaluation as a way to decrease cardiovascular risk in obese patients.

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Acquisition of data: Esposito, Pontillo, Di Palo, Masella, D. Giugliano.
Analysis and interpretation of data: Esposito, G. Giugliano, Marfella, D. Giugliano.
Drafting of the manuscript: Esposito, D. Giugliano.
Critical revision of the manuscript for important intellectual content: Esposito, Pontillo, Di Palo, G. Giugliano, Masella, Marfella, D. Giugliano.
Statistical expertise: Marfella, D. Giugliano.
Administrative, technical, or material support: Pontillo, Di Palo.
Study supervision: Esposito, G. Giugliano, Masella, D. Giugliano.

REFERENCES

1. Serdula MK, Mokdad AH, Williamson DF, et al. Prevalence of attempting weight loss and strategies for controlling weight. *JAMA*. 1999;282:1353-1358.
2. National Task Force on the Prevention and Treatment of Obesity. Overweight, obesity, and health risk. *Arch Intern Med*. 2000;160:898-904.
3. Flegal KM, Carroll MD, Ogden CL, Johnson CL. Prevalence and trends in obesity among US adults, 1999-2000. *JAMA*. 2002;288:1723-1727.
4. Ogden CL, Flegal KM, Carroll MD, Johnson CL. Prevalence and trends in overweight among US children and adolescents, 1999-2000. *JAMA*. 2002;288:1728-1732.
5. Kopelman PG. Obesity as a medical problem. *Nature*. 2000;404:635-643.
6. Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose tissue expression of tumor necrosis factor-α. *Science*. 1993;259:87-91.
7. Mohamed-Ali V, Goodrick S, Rawesh A, et al. Subcutaneous adipose tissue releases interleukin-6, but not tumor necrosis factor-α, in vivo. *J Clin Endocrinol Metab*. 1997;82:4196-4200.
8. Maeda K, Okubo K, Shimomuro I, et al. cDNA cloning and expression of a novel adipose specific collagen-like factor, apM1. *Biochem Biophys Res Commun*. 1996;221:286-289.
9. Bastard JP, Jardel C, Bruckert E, et al. Elevated levels of interleukin-6 are reduced in serum and subcutaneous adipose tissue of obese women after weight loss. *J Clin Endocrinol Metab*. 2000;85:3338-3342.
10. Ziccardi P, Nappo F, Giugliano G, et al. Reduction of inflammatory cytokine concentrations and improvement of endothelial functions in obese women after weight loss over one year. *Circulation*. 2002;105:804-809.
11. Esposito K, Pontillo A, Ciotola M, et al. Weight loss reduces interleukin-18 levels in obese women. *J Clin Endocrinol Metab*. 2002;87:3864-3866.
12. Yudkin JS, Stehouwer CD, Emeis JJ, et al. C-reactive protein in healthy subjects: associations with obesity, insulin resistance, and endothelial dysfunction. *Arterioscler Thromb Vasc Biol*. 1999;19:972-978.
13. Ridker PM, Hennekens CH, Buring JE, Rifai N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. *N Engl J Med*. 2000;342:836-843.
14. Harris TB, Ferrucci L, Tracy RP, et al. Associations of elevated interleukin-6 and C-reactive protein levels with mortality in the elderly. *Am J Med*. 1999;106:506-512.
15. Blankenberg S, Tiret L, Bickel C, et al. Interleukin-18 is a strong predictor of cardiovascular death in stable and unstable angina. *Circulation*. 2002;106:24-30.
16. Stefan N, Stumvoll M. Adiponectin—its role in metabolism and beyond. *Horm Metab Res*. 2002;34:469-474.
17. Robertson RM, Smaha L. Can a Mediterranean style diet reduce heart disease? *Circulation*. 2001;103:1821-1822.
18. Matthews DR, Hosker JP, Rudenski AS, et al. Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia*. 1985;28:412-419.
19. Corbi GM, Carbone S, Ziccardi P, et al. FFAs and QT intervals in obese women with visceral adiposity. *J Clin Endocrinol Metab*. 2002;87:2080-2083.
20. Freedman MR, King J, Kennedy E. Popular diets: a scientific review. *Obes Res*. 2001;9(suppl 1):1S-40S.
21. Stouthard JM, Romjin JA, Van Der Poll T, et al. Endocrinologic and metabolic effects of interleukin-6 in humans. *Am J Physiol*. 1995;268:E813-E819.
22. Yang W-S, Lee WJ, Funahashi T, et al. Weight reduction increases plasma levels of an adipose-derived anti-inflammatory protein, adiponectin. *J Clin Endocrinol Metab*. 2001;86:3815-3819.
23. Fruebis J, Tsao TS, Javorschi S, et al. Proteolytic cleavage product of a 30 Kd adipocyte complement-related protein increases fatty acid oxidation in muscle and causes weight loss in mice. *Proc Natl Acad Sci U S A*. 2001;98:2005-2010.
24. Egger G, Swinburn B. An ecological approach to the obesity pandemic. *BMJ*. 1997;315:477-480.
25. de Lorgeril M, Salen P, Martin I, et al. Mediterranean diet, traditional risk factors and the rate of cardiovascular complications after myocardial infarction. *Circulation*. 1999;99:779-785.
26. Singh RB, Dubnov G, Niaz MA, et al. Effect of Indo-Mediterranean diet on progression of coronary artery disease in high risk patients (Indo-Mediterranean Diet Heart Study). *Lancet*. 2002;360:1455-1461.
27. Blake GJ, Ridker PM. Novel clinical markers of vascular wall inflammation. *Circ Res*. 2001;89:763-771.
28. Kumada M, Kihara S, Sumitsuji S, et al. Association of hypoadiponectinemia with coronary artery disease in men. *Arterioscler Thromb Vasc Biol*. 2003;23:85-89.
29. Heilbronn LK, Noakes M, Clifton MP. Energy restriction and weight loss on very-low-fat diet reduces C-reactive protein concentrations in obese, healthy women. *Arterioscler Thromb Vasc Biol*. 2001;21:968-970.
30. Tchernof A, Nolan A, Sites CK, Ades PA, Poehlman ET. Weight loss reduces C-reactive protein levels in obese postmenopausal women. *Circulation*. 2002;105:564-569.

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