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Effect of a Reduced-Calorie Diet on Plasma Levels of Inflammatory and Metabolic Factors in Overweight/Obese Patients with Cardiovascular Risk Factors

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Abstract

Background: Calorie restriction without malnutrition is likely to improve cardiovascular risk factors.
Objectives: The aim of this study was to investigate calorie restriction on markers of cardiometabolic risk in overweight/obese adults with cardiovascular risk factors.
Methods: In a parallel controlled trial, patients with overweight or obesity and one or more cardiovascular risk factor were randomized to a modest reduced-calorie diet (75% of the total calculated energy requirements) or control (no calorie restriction) groups and followed up for two months. Body weight, dietary intake, fasting plasma levels of C-reactive protein (CRP), monocyte chemoattractant protein-1 (MCP-1), intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), brain-derived neurotrophic factor (BDNF), neuropeptide Y (NPY), lipids, and glycemic factors were measured at baseline, and after two months. The differences were analyzed with analysis of covariance (ANCOVA).
Results: Sixty-six participants (33 in each group) completed the study. Body weight changed in the reduced-calorie diet group (-3.05 ± 2.65 kg), and blood pressure was improved (systolic -6.96 ± 12.04 and diastolic - 3.90 ± 8.97 mmHg). The reduced-calorie diet improved plasma ICAM-1 (change from baseline - 0.45 ± 1.99 ng/mL, P = 0.033, ANCOVA), MCP-1 (change from baseline - 0.50 pg/mL, P = 0.011, ANCOVA), low-density lipoprotein cholesterol (change from baseline - 9.35 ± 19.61 mg/dL, P < 0.001, ANCOVA), and triglyceride (change from baseline -33.66 ± 49.08, P = 0.001, ANCOVA), but BDNF, NPY, and other cardiometabolic factors were not different.
Conclusions: In overweight/obese subjects with cardiovascular risk factors which have been under medical treatment with risk-reducing medications, a modest weight loss induced by a reduced-calorie diet improved lipid profile, blood pressure, and reduced ICAM-1 and MCP-1 levels but had no effect on plasma BDNF or glycemic factors.

Keywords: Calorie-Restriction, Obesity, Cardiometabolic

1. Background

Obesity is a globally growing public health challenge, and nearly two out of five adults are currently overweight or obese (1). The rising prevalence of overweight and obesity has multiple health and economic impacts (2). Epidemiological studies have consistently shown a positive correlation between excess body weight and the major cardiovascular risk factors and cardiovascular disease outcomes (3). Obesity is associated with a higher prevalence of comorbidities such as dyslipidemia, hypertension, metabolic syndrome, and type 2 diabetes mellitus (T2DM) (4).

Inflammation may play a critical role in the pathophysiology of obesity-related comorbidities. Obesity may result in dysregulation of immunity and creates a state of chronic low-grade inflammation (5). Furthermore, it may result in perivascular adipose tissue inflammation, which may promote insulin resistance in the vasculature and contribute to endothelial dysfunction (6). Some of the inflammation mediators, which originated from inflamed endothelial cells, are the key mediators of endothelial-leukocyte interactions that contribute to endothelial dysfunction and promote atherosclerosis (7). In addition to the known inflammatory mediators, other molecules, such as brain-derived neurotrophic

factor (BDNF), may be involved in this process. BDNF is an abundant neurotrophin originally discovered in the brain. Besides roles in neurons, accumulating evidence indicates that BDNF appears to have roles in cardiovascular disease (8). The brain-derived neurotrophic factor is present in the systemic circulation, where it is produced by various types of cells, including activated lymphocytes and monocytes (9) and vascular endothelial cells (10). Brain-derived neurotrophic factor and its receptors have been shown to stimulate angiogenesis and maintain vascular integrity (11). Furthermore, circulating BDNF has been reported to be negatively associated with adhesion molecules, including intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) (12). It also plays an important role in food intake regulation and weight control (13).

Calorie restriction with adequate intake of protein and micronutrients is likely to improve health in overweight and obese individuals (14) and has been proposed as a means to improve cardiovascular risk markers (15, 16). Low-grade chronic inflammation may increase the risk of developing insulin resistance, T2DM, and cardiovascular disease, and calorie restriction may exert an anti-inflammatory effect (16). Although the effects of calorie restriction and weight loss on inflammatory factors as well as on circulating BDNF have been investigated in previous research, they mainly concern overweight and obese healthy subjects, and relatively less research has been conducted on patients with overweight/obesity with cardiovascular risk factors which have been under medical treatment with risk-reducing medications. Furthermore, the debate on whether calorie restriction can alter the circulating BDNF level continues, as studies have shown an increase (17, 18) or a decrease (19) in circulating BDNF.

2. Objectives

The present study aimed to investigate the effect of a reduced-calorie diet on plasma inflammatory factors, metabolic factors, and BDNF in overweight/ obese subjects with one or more cardiovascular risk factors.

3. Methods

3.1. Study Design and Subjects

The present study was a randomized clinical trial. Potential participants with cardiovascular risk factors were recruited through advertisements in regional health centers. Inclusion criteria were adults who had body mass index (BMI) > 25 kg/m^2, who had no weight-loss diet for at least three months before participating in the study, and those who had one or more classical cardiovascular risk factors, including hypertension, diabetes mellitus and/or dyslipidemia. The patients' hypertension and diabetes were controlled, and their medications had not changed in the last three months. The participants were excluded if they had cancer, regularly used insulin, took antipsychotic, anticonvulsant drugs, or omega-3 supplements, o had creatinine > 1.4 mg/dL, or were in pregnancy and breastfeeding periods.

The study was performed in compliance with the Helsinki Declaration, and informed consent was obtained from the participants. The study protocol was approved by the Ethics Committee (National Nutrition and Food Technology Research Institute, Shahid Beheshti University of Medical Sciences, Tehran, Iran), and the ethical committee code was IR.SBMU.NNFTRI.REC.1400.082. This trial was registered at link: www.irct.ir/ (IRCT20160702028742N11, on 29/01/2022).

The participant was randomly allocated to either the control or reduced-calorie diet groups according to the randomization schedule. Stratified block randomization was used, and the participants were stratified based on their risk factors (four strata: (1) hypertension +, diabetes +; (2) hypertension +, diabetes -; (3) hypertension -, diabetes +; (4) hypertension -, diabetes -). The participants belonging to each stratum were then randomly assigned 1:1 to either of the groups by block randomization. A block size of four within each stratum was used (using computer-generated random numbers) between the two groups. Persons who measured laboratory outcomes and the investigators who analyzed data were blinded to the identity of the subjects to avoid biases.

In the control group, patients were given simple dietary advice to reduce salt intake, foods with high saturated fat content, simple sugars, and foods containing added simple sugars. In the reduced-calorie diet, the participants were prescribed a low-calorie diet. For this purpose, daily weight maintenance energy was estimated for each participant by Mifflin et al.'s equation (20) and the level of physical activity. The participants have prescribed energy intake deficits of 25% of the total calculated energy requirements. The energy was distributed as ~ 55% from carbohydrates, ~ 27% from fats, and ~ 18% from proteins, and the number of servings of each food group that a participant can consume daily was determined. To provide adequate micronutrients and protein intake, the inclusion of low-calorie, nutrient-dense foods such as vegetables, whole fruits, and legumes, as well as low-fat dairy, poultry, and lean cuts of meats in the diet, were considered.

All of the participants were asked not to take omega-3

or antioxidant supplements during the study. One week after entering the study, the participants were contacted to answer their questions related to diet. The dietary intervention lasted for two months. To ensure adherence to the dietary intervention, the participants were followed up every two weeks by telephone. The participants were asked to maintain their current physical activity levels throughout the study.

3.2. Data Collection

Baseline characteristics, dietary intake, physical activity level, body weight, blood pressure, and laboratory findings were collected. Outcomes were determined at baseline and after two months. Food intake was evaluated through face-to-face and/or telephone interviews using a 24-hour dietary recall questionnaire completed in three days (two regular workdays and one day at the weekend) at the baseline and after two months, and the was analyzed using the Nutritionist software (version IV, N-Squared Computing, CA, USA). Physical activity was assessed by the international physical activity questionnaire (IPAQ) (21). Body weight was measured by a balance beam scale in light street clothes. During each visit, after five minutes of rest, while the participant was seated, using a digital arm sphygmomanometer (Omron digital automatic blood pressure monitor HEM-907), two blood pressure measurements were obtained, and the mean was calculated.

Venous blood samples were obtained after 10 - 12 h overnight fasting in heparinized tubes, and plasma was separated by centrifugation and stored at - 80°C for later biochemical analysis. Plasma total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and triglycerides (TG) were measured in batches by commercial kits (Pars-Azmoon, Karaj, Iran) by an automated analyzer (Selectra ProXL, Vital Scientific, Spankeren, The Netherlands). Plasma low-density lipoprotein cholesterol (LDL-C) was calculated using the equation (LDL-C = TC - HDL-C- (TG/5). Plasma levels of C-reactive protein (CRP) were measured with a kit (Audit Diagnostics, Cork, Ireland) using an autoanalyzer. Commercial enzyme-linked immunosorbent assay (ELISA) kits were used to measure plasma levels of monocyte chemoattractant protein-1 (MCP-1), BDNF (Biolegend, San Diego, USA), insulin (Monobind, Inc., Lake Forest, CA, USA), ICAM-1, VCAM-1, plasminogen activator inhibitor-1 (PAI-1) and Neuropeptide Y (NPY) (R&D System, Minneapolis, MN). Homeostatic model assessment of insulin resistance (HOMA-IR) was calculated using glucose and insulin values:

$$HOMA - IR = \frac{Glucose \times Insulin}{405}$$

3.3. Statistical Analyses

The study sample size was calculated using circulating BDNF as the main outcome variable. By using mean and standard deviation (SD) of serum BDNF changes of men with metabolic syndrome from a previous study (22) (serum BDNF change from 40.4 ± 7.8 to 46.9 ± 8.9 ng/mL, $P < 0.001$) at $\alpha = 0.05$ with 80% power, 28 participants were required for each arm. Considering an attrition rate of 20%, 34 participants were required for each group.

Statistical analyses were carried out using SPSS 25.0 (IBM Corp.). Data normality was determined by Kolmogorov–Smirnov test. Data are expressed as the means ± SD for normally distributed data and as the medians (quartiles 1 and 3) for skewed continuous variables. Between-group comparisons of baseline values were performed using the chi-square test or independent *t*-test (or Mann-Whitney U test for skewed continuous variables). The per-protocol analysis was performed. Within-group comparisons were done by paired *t*-test (or Wilcoxon test for skewed variables). Group comparisons for outcome data were performed using analysis of covariance (ANCOVA) controlling for covariates, and skewed variables were log-transformed before use. All tests were two-tailed. $P < 0.05$ was considered significant.

4. Results

Among 68 patients who were randomized to the two groups (n = 34 in each group), 33 subjects in the reduced-calorie diet and 33 subjects in the control group completed the study (Figure 1). The participants' baseline characteristics are presented in Table 1. None of the patients who completed the study had an inflammatory or autoimmune disease. About 85% of the patients had hypertension, and 30% had diabetes mellitus. About 88% of the patients were taking statins. All patients with T2DM were taking oral hypoglycemic medications, and none were receiving exogenous insulin.

Table 2 shows calorie and macronutrient intake at baseline and month two. At baseline, calorie intake was not different between the two groups. Although carbohydrate intake was higher in the reduced-calorie diet group compared to the control group, the fat intake was lower. The diet assessment at the end of the study showed that both carbohydrate and fat intake were lower in the reduced-calorie diet group than in the control group.

At baseline, body weight, BMI, and physical activity were different between the two groups. After two months,

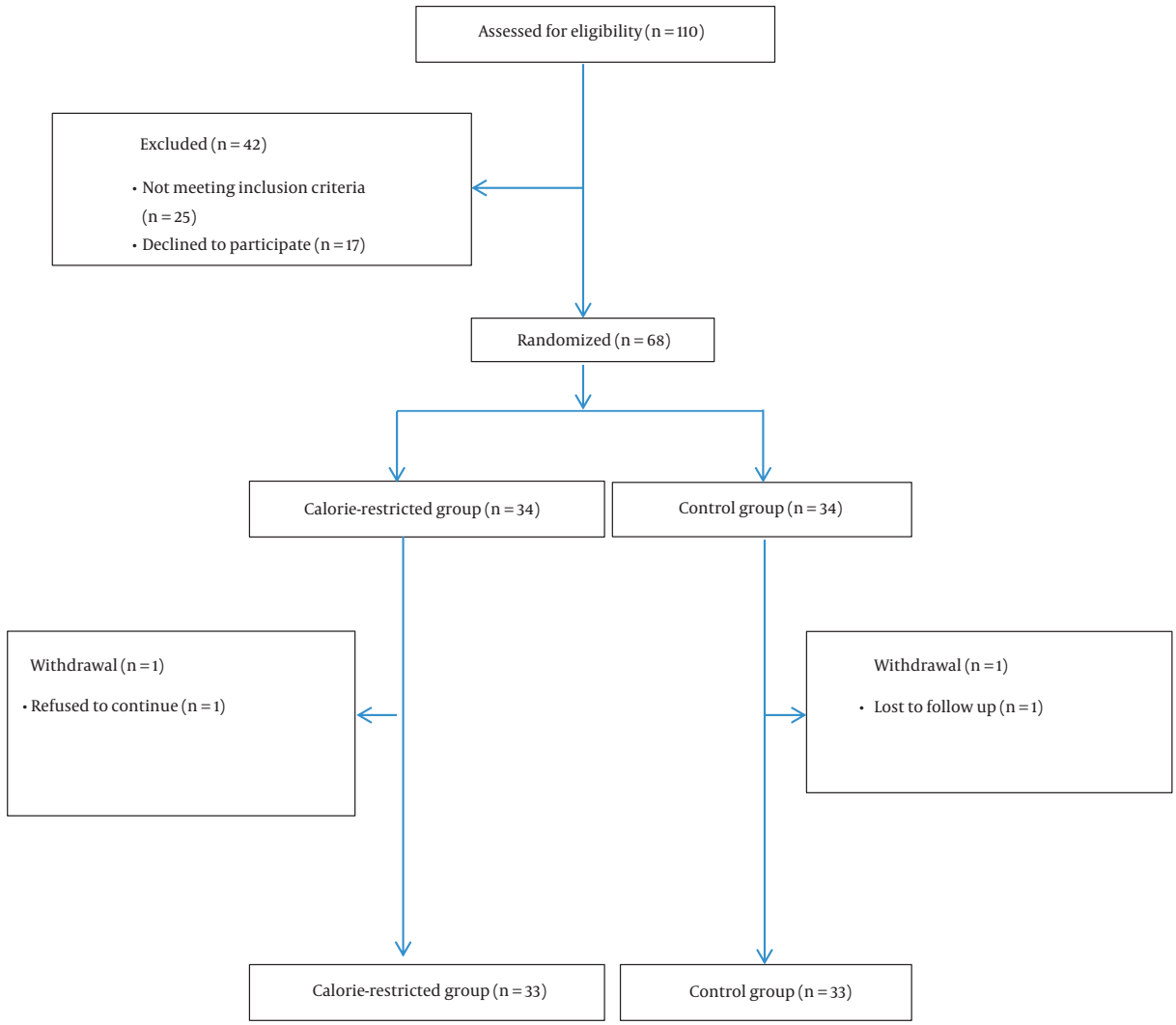

Figure 1. The consort flowchart of the study

body weight significantly decreased in the reduced-calorie diet (mean change - 3.05 ± 2.65 kg) with no changes in the control group (mean change 0.10 ± 0.73 kg), and the between-group difference was statistically significant ($P < 0.001$) (Table 3). There were no significant changes in physical activity levels within or between the two groups. Calorie restriction and the associated weight loss reduced both systolic (mean change - 6.96 ± 12.04 mmHg) and diastolic (mean change - 3.90 ± 8.97 mmHg) blood pressure in the reduced-calorie group, which were statistically significant from the control group ($P = 0.03$ and $P = 0.01$ for between-group differences of systolic and diastolic blood pressure, respectively).

Baseline plasma levels of TC, HDL-C, LDL-C, and non-HDL-C were significantly lower in the reduced-calorie diet than in the control group (Table 4). After two months, calorie restriction reduced plasma LDL-C (mean change - 9.35 ± 19.61 mg/dL) and TG (mean change - 33.66 ± 49.08 mg/dL), whereas no change in the LDL-C (mean change - 0.52 ± 26.91 mg/dL) and an increase in TG (mean change 30.63 ± 50.48) levels were observed in the control group. Calorie restriction reduced glucose, but there were no significant post-intervention differences in glucose and insulin. Plasma levels of NPY increased in the reduced-calorie diet group, but no between-group difference was observed after two months. Baseline plasma levels of BDNF were not significantly different between the two groups. There was no significant difference in plasma

Table 1. Baseline Characteristics of the Participants According to Study Groups [a, b]

Variables	Total (66)	Reduced-Calorie Diet (N = 33)	Control (N = 33)	P-Value
Age (y)	57.9 ± 7.9	59.4 ± 7.9	56.4 ± 7.7	0.12
Sex, male	29 (43.9)	18 (54.5)	11 (33.3)	0.08
History of diabetes mellitus	20 (30.3)	10 (30.3)	10 (30.3)	1.00
History of hypertension	56 (84.8)	28 (84.8)	28 (84.8)	1.00
Current smokers	3 (4.5)	0 (0)	3 (9.1)	0.11
Statin use	57 (88.3)	31 (93.9)	26 (79)	0.07
ACE-I/ARB use	39 (59.1)	22 (66.6)	17 (51.5)	0.21
Beta-blocker use	28 (42.4)	15 (45.4)	14 (42.4)	0.84
Calcium blocker use	10 (15.1)	6 (18.2)	4 (12.1)	0.49
Other hypertension medication use	16 (24.2)	10 (30.3)	6 (18.2)	0.25
Biguanides use	20 (30.3)	10 (30.3)	10 (30.3)	1.00
Sulfonylureas use	7 (10.6)	4 (12.1)	3 (9.1)	0.69
Other oral anti-hyperglycemic drugs use	3 (4.5)	2 (6.0)	1 (3.0)	0.55
Plasma creatinine (mg/dL)	1.16 ± 0.36	1.16 ± 0.48	1.17 ± 0.20	0.882
Body weight (kg)	80.15 ± 12.57	84.84 ± 13.29	75.46 ± 9.95	0.002
BMI (Kg/m^2)	29.98 ± 3.75	31.85 ± 4.18	28.17 ± 2.07	< 0.001

Abbreviations: ACE-I, angiotensin-converting-enzyme inhibitor; ARB, angiotensin receptor blocker; BMI, body mass index.
[a] Values are expressed as mean ± SD or No. (%).
[b] Values were analyzed using an independent *t*-test or chi-square test.

levels of BDNF between the two groups at the end of the study (Table 4).

Plasma levels of inflammatory markers and PAI-1 were not different at baseline, but there were between-group differences in ICAM-1 (P = 0.03, mean changes of - 0.45 ± 1.99, and 0.40 ± 1.25 ng/mL for the reduced-calorie and control groups, respectively) and MCP-1 (P = 0.01, mean changes of - 0.50 (- 11.25, 7.50) and 21.37 (- 1.81, 44.69) pg/mL for the reduced-calorie and control groups, respectively) levels after two months. No within or between-group differences were detected in concentrations of CRP, VCAM-1, or PAI-1 (Table 4).

5. Discussion

The results of the current study in overweight/obese subjects with one or more cardiovascular risk factors showed that modest calorie restriction for two months was associated with modest body weight reduction as well as improvements in plasma lipids, ICAM-1, and MCP-1. No significant changes were observed in plasma levels of BDNF, PAI-1, NPY, and glycemic markers.

At baseline, the weight and BMI of the patients in the reduced-calorie diet group were higher than the control group. This issue was probably one of the reasons for the higher baseline level of blood pressure and insulin resistance (HOMA-IR) in this group compared to the control group. Weight reduction in the calorie restriction group reduced blood pressure, plasma TC, TG, LDL-C, TC/HDL-C, and non-HDL-C. These findings were not unexpected because previous studies have consistently shown interventions that reduce body weight, including low-calorie diets, are well-established strategies to lower blood pressure and improve lipid profile (15). The baseline levels of TC and LDL-C were lower in the participants of the calorie restriction group, which may be partly due to the fact that more subjects from this group were treated with statins. In addition to weight loss, a lower calorie intake from fat in the reduced-calorie diet group compared to the control group may also have contributed to the lower baseline plasma LDL-C levels. Lower fat compared with higher fat diets may have a better effect on LDL-C levels (23). Regarding glycemic factors, although a within-group decrease in plasma glucose and HOMA-IR were observed by modest weight loss in the reduced-calorie group, no between-group differences were observed. We also performed analyses examining whether effects differed for those with and without diabetes; however, no significant effects were observed (data not shown). Subjects in the reduced-calorie

Table 2. Dietary Intake of the Participants at the Baseline and After Two Months [a, b]

Intake	Reduced-Calorie Diet (N = 33)	Control (N = 33)	P-Value
Energy (Kcal)			
Baseline	1819.78 ± 638.96	1824.58 ± 163.845	0.967
After 2 months	1510.55 ± 529.10	1813.22 ± 149.455	0.003
Carbohydrate (g)			
Baseline	260.19 ± 100.34	221.08 ± 39.06	0.041
After 2 months	210.52 ± 81.41	213.01 ± 31.74	0.871
Carbohydrate %			
Baseline	57.59 ± 9.65	48.73 ± 8.82	0.001
After 2 months	56.16 ± 9.33	47.25 ± 7.67	0.001
Protein (g)			
Baseline	77.15 ± 27.09	80.73 ± 28.09	0.601
After 2 months	68.39 ± 30.10	76.33 ± 24.31	0.243
Protein %			
Baseline	16.96 ± 12.38	17.59 ± 5.64	0.790
After 2 months	17.83 ± 4.47	16.74 ± 4.79	0.340
Fat (g)			
Baseline	52.36 ± 18.38	72.94 ± 16.81	0.001
After 2 months	43.86 ± 18.44	72.87 ± 17.32	0.001
Fat %			
Baseline	25.90 ± 15.78	35.81 ± 6.44	0.002
After 2 months	26.12 ± 7.85	36.00 ± 7.02	0.001
SFA (g)			
Baseline	13.74 ± 7.48	16.15 ± 5.28	0.136
After 2 months	13.23 ± 6.44	16.31 ± 3.74	0.022
SFA %			
Baseline	7.30 ± 3.92	7.94 ± 2.42	0.432
After 2 months	7.80 ± 3.11	8.10 ± 1.79	0.629
Fiber (g)			
Baseline	17.59 ± 9.74	16.28 ± 6.23	0.515
After 2 months	16.19 ± 8.91	15.84 ± 5.60	0.851

Abbreviation: SFA, saturated fatty acid.
[a] Values are expressed as mean ± SD.
[b] Values were analyzed using an independent *t*-test.

diet group had a relatively higher carbohydrate diet, which may have accounted for the lack of significant differences in glycemic factors. In a study of overweight or obese adults with T2DM, a low-calorie diet with lower carbohydrates (45% energy from carbohydrates) had lower mean glucose concentration and HbA1c than the low-calorie, high-carbohydrate diet (60% energy from carbohydrates) (24). However, contrary to the results of this study, in another study, a hypocaloric, high-carbohydrate diet (53% of energy as carbohydrates) compared to a hypocaloric, low-carbohydrate diet has resulted in comparable weight loss and improvement in glycemic control in patients with diabetes; however, the effects on glycemic variability indices were greater in the low-carbohydrate group (25).

Baseline NPY levels were higher in the reduced-calorie diet group than in the control group, which may be related to their higher body weight. It has been shown that obese

Table 3. Body Weight, Physical Activity, and Blood Pressure of the Participants [a, b]

Variables	Reduced-Calorie Diet (N = 33)	Control (N = 33)	P-Value
Weight (Kg)			< 0.001 [c]
Baseline	84.84 ± 13.29	75.46 ± 9.95 †	
After 2 months	81.78 ± 13.04 **	75.56 ± 9.69	
Change from baseline	-3.05 ± 2.65	0.10 ± 0.73	
BMI (Kg/m^2)			< 0.001 [c]
Baseline	31.85 ± 4.18	28.17 ± 2.07 †	
After 2 months	30.70 ± 4.07 **	28.22 ± 1.98	
Change from baseline	-1.15 ± 0.96	0.04 ± 0.27	
Physical activity (MET- hr/d)			0.091 [c]
Baseline	22.44 ± 4.44	24.52 ± 3.03 †	
After 2 months	22.49 ± 4.33	24.63 ± 3.22	
Change from baseline	0.04 ± 6.46	0.11 ± 1.60	
Systolic BP (mmHg)			0.036 [d]
Baseline	134.42 ± 18.32	127.54 ± 9.85	
After 2 months	127.45 ± 16.34 **	126.18 ± 8.27	
Change from baseline	-6.96 ± 12.04	-1.36 ± 3.87	
Diastolic BP (mmHg)			0.012 [d]
Baseline	80.75 ± 12.88	77.57 ± 9.37	
After 2 months	76.84 ± 10.30 *	78.85 ± 7.63	
Change from baseline	-3.90 ± 8.97	1.27 ± 3.64	

Abbreviations: BMI, body mass index; BP, blood pressure.
[a] Values are expressed as mean ± SD.
[b] Significantly different from baseline using paired *t*-test or Wilcoxon test (** P = 0.01, * P = 0.05). †: Significant difference between baseline values using independent *t*-test or Mann-Whitney U test.
[c] Values were analyzed using the ANCOVA test with baseline values of each variable.
[d] Values were analyzed using ANCOVA adjusted for baseline values of each variable, BMI, and physical activity as covariates.

adults have higher serum NPY levels than normal-weight adults (26). In the present study, calorie restriction increased NPY levels with no between-group differences. NPY is a potent orexigenic peptide that stimulates food intake (preferentially carbohydrate intake) and delays satiety with increased motivation to eat (27).

We could not detect a difference in plasma BDNF levels between the two groups, and weight reduction in the calorie restriction group did not change plasma BDNF levels. Despite frequent reports on the associations between body weight and circulating BDNF, a meta-analysis has found no association between BDNF levels and obesity (28). Our finding is in contrast to the findings in other human studies, which reported an increase in circulating BDNF after calorie-restriction induced-weight loss (17). Furthermore, serum BDNF level was increased following weight reduction through lifestyle modification in obese non-diabetic patients with schizophrenia (18). In contrast, reduced circulating BDNF levels have been reported following a very low-energy diet-induced weight loss (19). The lack of change in plasma BDNF in the current study is consistent with the finding of a study in which serum BDNF levels remained stable after one year of weight loss therapy in children and adolescents with obesity (29). The effect of diet-induced weight loss on plasma BDNF may be transient. Mohorko et al., in an uncontrolled intervention on obese adults, studied the effect of weight loss induced by a ketogenic diet on metabolic profile, including serum BDNF levels, and found that BDNF concentrations increased two weeks after starting the ketogenic diet but returned to baseline values in the eighth week (30).

Among the inflammatory biomarkers measured in the present study, ICAM-1 and MCP-1 concentrations were lower after calorie restriction but not that of VCAM-1 and CRP. The ICAM-1 is an adhesion molecule for leukocytes

on vascular endothelial tissue. This may indicate that the effect of modest weight loss on vascular inflammation is greater than its effects on systemic inflammation. The lack of change in VCAM-1 level despite the reduction in ICAM-1 level may be related to the biological effect of weight loss on endothelial cells. Obesity may cause an increase in shedding; thus, an increase in the circulating level of microparticles from different sources and weight loss is likely to be effective in reducing their level (31). It has been shown that microparticles interact with endothelial cells and increase the expression of ICAM-1 but not VCAM-1 (32). The observed decrease in ICAM-1, but not VCAM-1 concentration in the present study, is in line with the results of other diet-induced weight loss (33). In agreement, weight loss induced by caloric restriction has reduced circulating ICAM-1 (34). On the other hand, weight gain has increased circulating ICAM-1 level (35). Monocyte chemoattractant protein-1 is a pro-inflammatory chemokine produced by macrophages, endothelial cells, as well as adipocytes (7). Consistent with our findings, a low-calorie diet combined with lifestyle modification has prevented an increase in serum MCP-1 levels in women with metabolic syndrome (36). Furthermore, in a study within the PREDIMED-Plus trial, a 12-month intensive lifestyle intervention with an energy-restricted mediterranean diet produced weight loss and improved cardiovascular risk markers, including MCP-1, but not CRP in overweight/obese older adults with metabolic syndrome (37).

We did not observe a significant change in PAI-1 levels following weight loss in the calorie-reduced group. This effect is in contrast to the findings in other studies (31). The reason for this discrepancy may be related to the lower weight loss in this study since previous studies in which weight loss was associated with a decrease in PAI-1 had a greater weight reduction (31).

The present study had some limitations; therefore, it should be interpreted cautiously. This was a short-term trial of two months, and the study would have benefited if continued for a longer duration to assess long-term effects. Furthermore, the sample size was relatively small, and the power may be inadequate to detect subtle effects. The strength of the present study is that it was conducted in free-living adults in their usual living environment with a pragmatic reduced-calorie diet.

5.1. Conclusions

Modest weight loss induced by a reduced-calorie diet improved lipid profile, blood pressure, and reduced ICAM-1 and MCP-1 levels but did not affect glycemic factors and BDNF in overweight/obese adults with cardiovascular risk factors who have been under medical treatment with risk-reducing medications. By improving cardiovascular risk factors, the benefits of low-calorie-induced weight loss go beyond the success of weight loss. By ameliorating some pro-inflammatory factors, it may also affect other clinical conditions in which a higher BMI may contribute to disease course and activity. Further studies may help to better elucidate the possible role of BDNF in vascular inflammation in healthy subjects as well as in patients with cardiovascular disease.

Footnotes

Authors' Contribution: The research was conceived and designed by J. N; conducted by N. K, and M. M; data were analyzed and interpreted by J. N; the paper was written by J. N.

Clinical Trial Registration Code: IRCT20160702028742N11, on 29/01/2022.

Conflict of Interests: All authors declare that they have no conflict of interests.

Ethical Approval: The study protocol was approved by the Ethics Committee (National Nutrition and Food Technology Research Institute, Shahid Beheshti University of Medical Sciences, Tehran, Iran), and the Ethics Code was IR.SBMU.NNFTRI.REC.1400.082.

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Informed Consent: Informed consent was obtained from the participants.

References

1. N. C. D. Risk Factor Collaboration. Worldwide trends in body-mass index, underweight, overweight, and obesity from 1975 to 2016: A pooled analysis of 2416 population-based measurement studies in 128.9 million children, adolescents, and adults. *Lancet*. 2017;**390**(10113):2627–42. [PubMed ID: 29029897]. [PubMed Central ID: PMC5735219]. https://doi.org/10.1016/S0140-6736(17)32129-3.
2. Okunogbe A, Nugent R, Spencer G, Powis J, Ralston J, Wilding J. Economic impacts of overweight and obesity: Current and future estimates for 161 countries. *BMJ Glob Health*. 2022;**7**(9). [PubMed ID: 36130777]. [PubMed Central ID: PMC9494015]. https://doi.org/10.1136/bmjgh-2022-009773.
3. Dwivedi AK, Dubey P, Cistola DP, Reddy SY. Association between obesity and cardiovascular outcomes: Updated evidence from meta-analysis studies. *Curr Cardiol Rep*. 2020;**22**(4):25. [PubMed ID: 32166448]. https://doi.org/10.1007/s11886-020-1273-y.
4. Nguyen NT, Magno CP, Lane KT, Hinojosa MW, Lane JS. Association of hypertension, diabetes, dyslipidemia, and metabolic syndrome with obesity: Findings from the national

health and nutrition examination survey, 1999 to 2004. *J Am Coll Surg*. 2008;**207**(6):928–34. [PubMed ID: 19183541]. https://doi.org/10.1016/j.jamcollsurg.2008.08.022.
5. Cao H. Adipocytokines in obesity and metabolic disease. *J Endocrinol*. 2014;**220**(2):T47–59. [PubMed ID: 24403378]. [PubMed Central ID: PMC3887367]. https://doi.org/10.1530/JOE-13-0339.
6. Lastra G, Manrique C. Perivascular adipose tissue, inflammation and insulin resistance: Link to vascular dysfunction and cardiovascular disease. *Horm Mol Biol Clin Investig*. 2015;**22**(1):19–26. [PubMed ID: 25941914]. https://doi.org/10.1515/hmbci-2015-0010.
7. Engin A. Endothelial dysfunction in obesity. *Adv Exp Med Biol*. 2017;**960**:345–79. [PubMed ID: 28585207]. https://doi.org/10.1007/978-3-319-48382-5_15.
8. Hsu CY, Sheu WH, Lee IT. Brain-derived neurotrophic factor reduces long-term mortality in patients with coronary artery disease and chronic kidney disease. *Front Cardiovasc Med*. 2022;**9**:881441. [PubMed ID: 35800175]. [PubMed Central ID: PMC9253370]. https://doi.org/10.3389/fcvm.2022.881441.
9. Kerschensteiner M, Gallmeier E, Behrens L, Leal VV, Misgeld T, Klinkert WE, et al. Activated human T cells, B cells, and monocytes produce brain-derived neurotrophic factor in vitro and in inflammatory brain lesions: A neuroprotective role of inflammation? *J Exp Med*. 1999;**189**(5):865–70. [PubMed ID: 10049950]. [PubMed Central ID: PMC2192942]. https://doi.org/10.1084/jem.189.5.865.
10. Nakahashi T, Fujimura H, Altar CA, Li J, Kambayashi J, Tandon NN, et al. Vascular endothelial cells synthesize and secrete brain-derived neurotrophic factor. *FEBS Lett*. 2000;**470**(2):113–7. [PubMed ID: 10734218]. https://doi.org/10.1016/s0014-5793(00)01302-8.
11. Usui T, Naruo A, Okada M, Hayabe Y, Yamawaki H. Brain-derived neurotrophic factor promotes angiogenic tube formation through generation of oxidative stress in human vascular endothelial cells. *Acta Physiol (Oxf)*. 2014;**211**(2):385–94. [PubMed ID: 24612679]. https://doi.org/10.1111/apha.12249.
12. Xia F, Zeng Q, Chen J. Circulating brain-derived neurotrophic factor dysregulation and its linkage with lipid level, stenosis degree, and inflammatory cytokines in coronary heart disease. *J Clin Lab Anal*. 2022;**36**(7). e24546. [PubMed ID: 35666604]. [PubMed Central ID: PMC9279961]. https://doi.org/10.1002/jcla.24546.
13. Marosi K, Mattson MP. BDNF mediates adaptive brain and body responses to energetic challenges. *Trends Endocrinol Metab*. 2014;**25**(2):89–98. [PubMed ID: 24361004]. [PubMed Central ID: PMC3915771]. https://doi.org/10.1016/j.tem.2013.10.006.
14. Perry CA, Gadde KM. The role of calorie restriction in the prevention of cardiovascular disease. *Curr Atheroscler Rep*. 2022;**24**(4):235–42. [PubMed ID: 35107761]. https://doi.org/10.1007/s11883-022-00999-8.
15. Hasan B, Nayfeh T, Alzuabi M, Wang Z, Kuchkuntla AR, Prokop LJ, et al. Weight loss and serum lipids in overweight and obese adults: A systematic review and meta-analysis. *J Clin Endocrinol Metab*. 2020;**105**(12). [PubMed ID: 32954416]. https://doi.org/10.1210/clinem/dgaa673.
16. Kokten T, Hansmannel F, Ndiaye NC, Heba AC, Quilliot D, Dreumont N, et al. Calorie restriction as a new treatment of inflammatory diseases. *Adv Nutr*. 2021;**12**(4):1558–70. [PubMed ID: 33554240]. [PubMed Central ID: PMC8321869]. https://doi.org/10.1093/advances/nmaa179.
17. Araya AV, Orellana X, Espinoza J. Evaluation of the effect of caloric restriction on serum bdnf in overweight and obese subjects: Preliminary evidences. *Endocrine*. 2008;**33**(3):300–4. [PubMed ID: 19012000]. https://doi.org/10.1007/s12020-008-9090-x.
18. Kuo FC, Lee CH, Hsieh CH, Kuo P, Chen YC, Hung YJ. Lifestyle modification and behavior therapy effectively reduce body weight and increase serum level of brain-derived neurotrophic factor in obese non-diabetic patients with schizophrenia. *Psychiatry Res*. 2013;**209**(2):150–4. [PubMed ID: 23219101]. https://doi.org/10.1016/j.psychres.2012.11.020.
19. Glud M, Christiansen T, Larsen LH, Richelsen B, Bruun JM. Changes in circulating bdnf in relation to sex, diet, and exercise: A 12-week randomized controlled study in overweight and obese participants. *J Obes*. 2019;**2019**:4537274. [PubMed ID: 31781387]. [PubMed Central ID: PMC6875316]. https://doi.org/10.1155/2019/4537274.
20. Mifflin MD, St Jeor ST, Hill LA, Scott BJ, Daugherty SA, Koh YO. A new predictive equation for resting energy expenditure in healthy individuals. *Am J Clin Nutr*. 1990;**51**(2):241–7. [PubMed ID: 2305711]. https://doi.org/10.1093/ajcn/51.2.241.
21. Craig CL, Marshall AL, Sjostrom M, Bauman AE, Booth ML, Ainsworth BE, et al. International physical activity questionnaire: 12-country reliability and validity. *Med Sci Sports Exerc*. 2003;**35**(8):1381–95. [PubMed ID: 12900694]. https://doi.org/10.1249/01.MSS.0000078924.61453.FB.
22. Lee IT, Lee WJ, Tsai IC, Liang KW, Lin SY, Wan CJ, et al. Brain-derived neurotrophic factor not associated with metabolic syndrome but inversely correlated with vascular cell adhesion molecule-1 in men without diabetes. *Clin Chim Acta*. 2012;**413**(9-10):944–8. [PubMed ID: 22374129]. https://doi.org/10.1016/j.cca.2012.02.013.
23. Lu M, Wan Y, Yang B, Huggins CE, Li D. Effects of low-fat compared with high-fat diet on cardiometabolic indicators in people with overweight and obesity without overt metabolic disturbance: A systematic review and meta-analysis of randomised controlled trials. *Br J Nutr*. 2018;**119**(1):96–108. [PubMed ID: 29212558]. https://doi.org/10.1017/S0007114517002902.
24. Rock CL, Flatt SW, Pakiz B, Taylor KS, Leone AF, Brelje K, et al. Weight loss, glycemic control, and cardiovascular disease risk factors in response to differential diet composition in a weight loss program in type 2 diabetes: A randomized controlled trial. *Diabetes Care*. 2014;**37**(6):1573–80. [PubMed ID: 24760261]. [PubMed Central ID: PMC4392939]. https://doi.org/10.2337/dc13-2900.
25. Tay J, Luscombe-Marsh ND, Thompson CH, Noakes M, Buckley JD, Wittert GA, et al. Comparison of low- and high-carbohydrate diets for type 2 diabetes management: A randomized trial. *Am J Clin Nutr*. 2015;**102**(4):780–90. [PubMed ID: 26224300]. https://doi.org/10.3945/ajcn.115.112581.
26. Rahmanian M, Lotfi Yaghin N, Alizadeh M. Blood level of 2-arachidonoyl glycerol (2-AG), neuropeptide y and omentin and their correlation with food habits in obese women. *Galen Med J*. 2020;**9**. e1721. [PubMed ID: 34466576]. [PubMed Central ID: PMC8343500]. https://doi.org/10.31661/gmj.v9i0.1721.
27. Beck B. Neuropeptide Y in normal eating and in genetic and dietary-induced obesity. *Philos Trans R Soc Lond B Biol Sci*. 2006;**361**(1471):1159–85. [PubMed ID: 16874931]. [PubMed Central ID: PMC1642692]. https://doi.org/10.1098/rstb.2006.1855.
28. Sandrini L, Di Minno A, Amadio P, Ieraci A, Tremoli E, Barbieri SS. Association between obesity and circulating brain-derived neurotrophic factor (BDNF) levels: Systematic review of literature and meta-analysis. *Int J Mol Sci*. 2018;**19**(8). [PubMed ID: 30081509]. [PubMed Central ID: PMC6121551]. https://doi.org/10.3390/ijms19082281.
29. Makhout S, Vermeiren E, Van De Maele K, Bruyndonckx L, De Winter BY, Van Hoorenbeeck K, et al. The role of brain-derived neurotrophic factor in obstructive sleep apnea and endothelial function in a pediatric population with obesity. *Front Med (Lausanne)*. 2021;**8**:835515. [PubMed ID: 35127775]. [PubMed Central ID: PMC8811024]. https://doi.org/10.3389/fmed.2021.835515.
30. Mohorko N, Cernelic-Bizjak M, Poklar-Vatovec T, Grom G, Kenig S, Petelin A, et al. Weight loss, improved physical performance, cognitive function, eating behavior, and metabolic profile in a 12-week ketogenic diet in obese adults. *Nutr Res*. 2019;**62**:64–77. [PubMed ID: 30803508]. https://doi.org/10.1016/j.nutres.2018.11.007.
31. Morel O, Luca F, Grunebaum L, Jesel L, Meyer N, Desprez D, et al. Short-term very low-calorie diet in obese females

improves the haemostatic balance through the reduction of leptin levels, PAI-1 concentrations and a diminished release of platelet and leukocyte-derived microparticles. *Int J Obes (Lond)*. 2011;**35**(12):1479–86. [PubMed ID: 21386797]. https://doi.org/10.1038/ijo.2011.19.

32. Fu Z, Zhou E, Wang X, Tian M, Kong J, Li J, et al. Oxidized low-density lipoprotein-induced microparticles promote endothelial monocyte adhesion via intercellular adhesion molecule 1. *Am J Physiol Cell Physiol*. 2017;**313**(5):C567–74. [PubMed ID: 28814403]. https://doi.org/10.1152/ajpcell.00158.2016.
33. Sanchez E, Santos MD, Nunez-Garcia M, Bueno M, Sajoux I, Yeramian A, et al. Randomized clinical trial to evaluate the morphological changes in the adventitial vasa vasorum density and biological markers of endothelial dysfunction in subjects with moderate obesity undergoing a very low-calorie ketogenic diet. *Nutrients*. 2021;**14**(1). [PubMed ID: 35010908]. [PubMed Central ID: PMC8746664]. https://doi.org/10.3390/nu14010033.
34. Meydani SN, Das SK, Pieper CF, Lewis MR, Klein S, Dixit VD, et al. Long-term moderate calorie restriction inhibits inflammation without impairing cell-mediated immunity: A randomized controlled trial in non-obese humans. *Aging (Albany NY)*. 2016;**8**(7):1416–31. [PubMed ID: 27410480]. [PubMed Central ID: PMC4993339]. https://doi.org/10.18632/aging.100994.
35. Cronin BE, Allsopp PJ, Slevin MM, Magee PJ, McCaffrey TA, Livingstone MBE, et al. The effect of weight change over a 2-year period on inflammatory status in postmenopausal women. *Eur J Clin Nutr*. 2018;**72**(3):388–93. [PubMed ID: 29167576]. https://doi.org/10.1038/s41430-017-0014-9.
36. Oh EG, Bang SY, Kim SH, Hyun SS, Chu SH, Jeon JY, et al. Therapeutic lifestyle modification program reduces plasma levels of the chemokines CRP and MCP-1 in subjects with metabolic syndrome. *Biol Res Nurs*. 2013;**15**(1):48–55. [PubMed ID: 21859748]. https://doi.org/10.1177/1099800411416637.
37. Salas-Salvado J, Diaz-Lopez A, Ruiz-Canela M, Basora J, Fito M, Corella D, et al. Effect of a lifestyle intervention program with energy-restricted mediterranean diet and exercise on weight loss and cardiovascular risk factors: One-year results of the PREDIMED-plus trial. *Diabetes Care*. 2019;**42**(5):777–88. [PubMed ID: 30389673]. https://doi.org/10.2337/dc18-0836.

Table 4. Plasma Levels of Cardiometabolic Factors in the Participant of the Calorie Restriction and Control Groups at the Baseline and After Two Months [a, b]

Variables	Reduced-Calorie Diet (N=33)	Control (N=33)	P-Value [c]
TC (mg/dL)			< 0.001
Baseline	144.12 ± 32.47	181.81 ± 30.98 †	
After 2 months	127.30 ± 22.77**	185.96 ± 33.49	
Change from baseline	-16.81 ± 25.67	4.15 ± 31.65	
LDL-C (mg/dL)			< 0.001
Baseline	72.92 ± 24.73	109.36 ± 26.12 †	
After 2 months	63.56 ± 17.40 *	108.84 ± 27.38	
Change from baseline	- 9.35 ± 19.61	- 0.52 ± 26.91	
HDL-C (mg/dL)			0.114
Baseline	40.84 ± 8.70	46.90 ± 9.10 †	
After 2 months	40.15 ± 7.09	45.45 ± 9.26	
Change from baseline	- 0.69 ± 5.37	- 1.45 ± 5.32	
TC/HDL-C			< 0.001
Baseline	3.60 ± 0.85	3.96 ± 0.79	
After 2 months	3.23 ± 0.66 **	4.20 ± 0.89	
Change from baseline	- 0.37 ± 0.56	0.23 ± 0.75	
Non-HDL-C (mg/dL)			< 0.001
Baseline	103.24 ± 29.31	134.90 ± 28.10 †	
After 2 months	87.15 ± 20.65 **	140.51 ± 31.81	
Change from baseline	- 16.09 ± 23.20	5.60 ± 30.94	
TG (mg/dL)			0.001
Baseline	151.48 ± 67.16	127.72 ± 44.63	
After 2 months	117.81 ± 44.89 **	158.36 ± 62.76 **	
Change from baseline	- 33.66 ± 49.08	30.63 ± 50.48	
Glucose (mg/dL)			0.890 [d]
Baseline	100.00 (86.50, 117.50)	81.00 (73.50, 88.00) †	
After 2 months	95.00 (84.00, 107.50) *	81.00 (73.50, 92.00)	
Change from baseline	- 7.00 (- 11.00, 1.50)	2.00 (- 3.50, 9.00)	
Insulin (μIU/mL)			0.729
Baseline	9.65 ± 4.34	8.03 ± 4.17	
After 2 months	8.69 ± 3.67	8.40 ± 4.18	
Change from baseline	- 0.96±3.28	0.37.4.21	
HOMA-IR			0.727
Baseline	2.57 ± 1.24	1.64 ± 0.96 †	
After 2 months	2.21 ± 1.15	1.71 ± 0.86	
Change from baseline	- 0.35 ± 1.10	0.07 ± 0.98	
NPY (ng/mL)			0.409 [d]
Baseline	8.01 (5.84, 9.85)	5.37 (3.99, 7.02) †	

After 2 months	8.54 (5.65, 11.14) *	5.79 (4.94, 6.40)	
Change from baseline	0.53 (- 0.08, 0.90)	0.41 (- 0.45, 0.50)	
BDNF (pg/mL)			0.739
Baseline	1892.87 ± 1811.57	2303.45 ± 1242.71	
After 2 months	1944.63 ± 1698.99	2011.67 ± 1248.85	
Change from baseline	51.75 ± 1377.26	- 291.78 ± 1687.05	
CRP (mg/L)			0.603
Baseline	2.81 ± 2.12	2.33 ± 1.02	
After 2 months	2.46 ± 1.57	2.33 ± 1.04	
Change from baseline	- 0.35 ± 1.89	- 0.01 ± 1.12	
MCP-1 (pg/mL)			0.011 [d]
Baseline	81.55 (74.80, 112.55)	76.85 (63.31, 125.46)	
After 2 months	79.00 (71.30, 105.30)	109.97 (65.99, 157.69) **	
Change from baseline	- 0.50 (- 11.25, 7.50)	21.37 (- 1.81, 44.69)	
ICAM-1 (ng/mL)			0.030
Baseline	18.16 ± 2.87	18.33 ± 2.80	
After 2 months	17.71 ± 3.39	18.73 ± 2.84	
Change from baseline	- 0.45 ± 1.99	0.40 ± 1.25	
VCAM-1 (ng/mL)			0.996
Baseline	26.40 ± 0.83	26.39 ± 1.85	
After 2 months	26.59 ± 0.79	26.60 ± 1.96	
Change from baseline	0.19 ± 1.08	0.21 ± 1.47	
PAI-1 (ng/mL)			0.799 [d]
Baseline	2.73 (1.42, 5.51)	2.92 (0.70, 6.79)	
After 2 months	3.07 (0.85, 5.61)	2.09 (0.58, 6.06)	
Change from baseline	- 0.41 (- 1.34, 0.87)	- 0.21 (- 1.88, 0.65)	

Abbreviations: BDNF, brain-derived neurotrophic factor; BP, blood pressure; CRP, C-reactive protein; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment for insulin resistance; ICAM-1, intercellular adhesion molecule 1; LDL-C, low-density lipoprotein cholesterol; MCP-1, monocyte chemoattractant protein-1; NPY, neuropeptide Y; PAI-1, plasminogen activator inhibitor-1; TC, total cholesterol; TG, triglyceride.

[a] Values are expressed as mean ± SD or median (quartile 1, quartile 3).

[b] Significantly different from baseline using paired *t*-test or Wilcoxon test (** P = 0.01, * P = 0.05). †: Significant difference between baseline values using independent *t*-test or Mann-Whitney U test.

[c] Values were analyzed using ANCOVA adjusted for baseline values of each variable, BMI, and physical activity as covariates.

[d] Logarithmical transformation was done before ANCOVA.