Investigating the effects of negative-calorie diet compared with low-calorie diet under exercise conditions on weight loss and lipid profile in overweight/obese middle-aged and older men

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1. Introduction
The prevalence of abnormal weight gain is increasing worldwide. Obesity is associated with an increased prevalence of chronic diseases, including type 2 diabetes, hypertension, and cardiovascular disease (CVD) (1).

About 70% of middle-aged and older populations (aged ≥45 years) have abnormal weight gain. This population is under increased risk for obesity (2).

Physical dysfunctions and higher healthcare costs, as well as increased morbidity and mortality, are some consequences of obesity among elderly adults (3).

Today, weight-loss medications and diets are widely used for weight loss and body fitness. These things are associated with several side effects influencing the health of the person (4). Exercise alone without any dietary intervention has low efficacy in weight loss. Previous studies have shown that there is a direct relation between nutrition and body condition, (5,6). Therefore, health experts stress the importance of a low-calorie diet (LCD). However, the negative-calorie diet (NCD) has recently gained a great deal of research and popular attention.

The NCD is a relatively trendy diet based on the principle of consuming foods that create a negative caloric effect, with the aim of leading to appropriate weight loss. For example, for digesting a piece of dessert containing 400 calories, the body needs 150 calories of energy. The remaining 250 calories add to the body fat. The NCD consists of foods that require 150 calories to digest only 100 calories’ worth of food (instead of 400); thus, the body should burn 50 extra calories simply by eating the food. This idea for weight loss is appealing. This gives these foods a natural, fat-burning property. These foods are called catabolic foods. According to the claims of the NCD's supporters, this diet will contribute more than other diets to weight loss. In other words, more eating reduces more weight (7).
The origin of the NCD idea is still unclear. The notion first appeared on the website www.negativecaloriediet.com as an 80-page downloadable e-book in 2007. Nowadays, there are many advertisements about the NCD. Notwithstanding the popularity of the NCD idea among dietary plans, there has been no scientific evidence about the efficacy and effects of this diet on weight loss. Therefore, the present study aimed to evaluate the effects of the NCD supplemented with exercise on weight loss and to compare its efficacy with the LCD with exercise. Changes in lipid profiles of the subjects, body weight, body mass index (BMI), high-density lipoprotein (HDL) cholesterol levels, and total cholesterol to HDL cholesterol ratios were assessed prior to and after intervention to assess weight-loss trends.

2. Materials and methods
This was a randomized clinical trial study. Participants of the study (n = 37) were randomly selected among overweight or obese men aged 45–75 years who had attended (during the 2 months prior to the study) the Ukrainian Center of Sports Medicine in Kiev to attend weight-loss consulting programs. The research team consisted of 3 physicians (2 sport physicians and 1 resident) and 2 nurses. Enrollment, classification, and assignment of subjects to experiments were all done under the supervision of a sports medicine physician. Participants were nonsmokers and weight-stable (±2 kg for more than 1 year) with no history of regular exercise in at least 3 months before the study. They had no history of CVD or other disorders such as diabetes, depression, eating disorders, chronic medication usage, kidney disease, cancer, food allergies, or intolerances to items used in meals. Subjects with abnormalities in the thyroid or their electrocardiographs, or any history of antiobesity medication, weight-loss drugs, or dietary supplementations for weight control, were excluded from the study.

The participants were assessed for vital signs and their blood pressure was measured from the right arm (twice, 5-min intervals) with a manual mercury sphygmomanometer after a 10-min sitting rest period. The average value of 3 measurements was used for further analysis (as shown in Table 2). Those subjects with blood pressures lower than 140/90 mmHg were entered into the experiments.

Weight-loss assessment parameters included body weight, BMI, and blood lipid parameters, which were measured prior to and after intervention.

BMI was calculated as body weight (kg) / height (m²); a BMI equal to or greater than 25.0 kg/m² was defined as overweight and obese. Height was measured to the nearest 0.1 cm by a wall-mounted stadiometer. Body weight was measured to the nearest 0.1 kg on a digital scale (Sacle-Tronix model 5002, USA). All physical measurements were performed with light street clothing and no shoes.

Blood samples were taken from the antecubital vein. Total cholesterol (total C), triglycerides (TG), and HDL cholesterol (HDL-C) were measured by spectrophotometry at 500 nm using an enzymatic kit (Elitech Diagnostics, France). Low-density lipoprotein (LDL) cholesterol (LDL-C) was calculated using the Friedewald formula, defined as LDL-C = total C – HDL-C – TG/5 (8). The participants did not eat or drink, except water, for 9–12 h prior to the blood test. Blood sample assessments for further analyses were as follows: plasma concentration of total C below 200 mg/dL, LDL-C below 130 mg/dL, TG below 150 mg/dL, and, finally, plasma HDL-C levels below 40–60 mg/dL.

Following initial assessments, the participants were randomly divided into 2 groups according to a simple randomized study design. There were no restrictions such as blocking and block size, though there was a balance between the study groups in terms of size and baseline characteristics (9). All groups received special recipes developed with the Food Guide Pyramid and Dietary Guidelines (United States Department of Agriculture), the UK Food Standards Agency, and the NCD plan (10,11). Study group I (18 participants) received the NCD (details of diet: 15% protein, 75% carbohydrates, 10% fat) with exercise (NCDsport). The NCD is a low-fat, high-carbohydrate, and high-fiber diet (7,12,13). It is believed that negative-calorie foods promote weight loss by burning more calories than the foods contain. Study group II (19 participants) received the LCD (details of diet: 15% protein, 55% carbohydrates, 30% fat) with exercise (LCDsport). In general, a LCD is high in carbohydrates and

<table>
<thead>
<tr>
<th>Group</th>
<th>Type of diet</th>
<th>BMR × PAL</th>
<th>Daily energy intake* (kcal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (NCD)</td>
<td>Negative-calorie diet</td>
<td>2863 ± 236</td>
<td>2734 ± 201</td>
</tr>
<tr>
<td>II (LCD)</td>
<td>Low-calorie diet</td>
<td>2827 ± 211</td>
<td>2720 ± 189</td>
</tr>
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</table>

*: Daily energy intake = (BMR × PAL) – 5%.
low in fat, (7,14). Low-calorie foods contribute to weight loss by reducing total caloric intake, but they do not help to burn calories. A large part of the NCD includes low-calorie fruits and vegetables that are high in fiber (15,16; http://content.time.com/time/specials/packages/article/0,28804,1896439_1896359_1896346,00.html). The NCD includes more than 100 different foods. These negative-calorie foods consisted of lean protein such as poultry, red meat, fish, eggs, vegetables, and fruits. Fruits consisted of apple, blueberry, cranberry, grapefruit, honeydew, lemon/lime, mango, orange, papaya, pineapple, strawberry, tangerine, and watermelon. Poultry sources included chicken and turkey breast. Red meat consisted of top round, extra lean sirloin, and game meats. Fish sources included all varieties, such as buffalo fish, catfish, clams (raw or cooked), cod steaks, crab, crayfish, flounder, mussels, oysters (cooked or half shell), shrimp, trout, and tuna. Eggs included egg whites and whole eggs in moderate quantities, but at least about 1 yolk a day. Vegetables consisted of asparagus, bean sprouts, beetroot, broccoli, cabbage, carrot, cauliflower, celery, cucumber, green beans, kale, leek, lettuce, radish, spinach, tomato, and turnip. The low-calorie foods list, also as described above, included low-fiber fruits and vegetables and other foods that were not described above.

Participants used a diary that was previously approved based on household measures. Diaries were checked for completeness and energy, and macronutrient compositions were calculated using the ‘Diets In Details’ software.

Both groups had a 5% caloric restriction from their maintenance energy requirements and a 10% increase in energy expenditure through structured regular exercise. Table 1 shows daily caloric needs and maintenance caloric requirements, estimated by multiplying the basal metabolic rate (BMR) and physical activity level (PAL) of the subject. For a more accurate estimate of BMR in men, Eq. (1) and Eq. (2) were used for the participants 31–60 years old and those more than 60 years old, respectively (11).

Eq. (1): (Weight in kg × 11.6) + 879
Eq. (2): (Weight in kg × 13.5) + 487

The PAL is the ratio of total daily energy expenditure to BMR. The PAL value was classified into 1 of 7 categories as follows: not at all, less than once a month, 1–2 times a month, 1 time a week, 2–3 times a week, 4–5 times a week, and every day. The exercise frequency of 1–2 times a month or less was considered as continuously inactive. At baseline, for selecting participants with a sedentary lifestyle, the PAL values were determined using a customized self-report questionnaire. The questionnaire consisted of a 7-point Likert-type scale ranging from “not at all” (1 point) to “every day” (7 points) (10,17).

During the study, the participants had an active lifestyle (exercise sessions more than 3 times a week), and the PAL was considered to be 1.5. To assess weight loss at a healthy and effective rate, we considered a 15% reduction (10% of energy expenditure and 5% of caloric restriction) in the maintenance calorie needs (11). Participants used a suitable method for identifying their diet and beverage habits. They recorded food and beverage consumption (including water) for 4 days a week (3 weekdays + 1 weekend day). They did it at the start of the study (baseline) and every month during the study.

Energy expenditure increased equally in both groups as they underwent supervised exercise 5 days a week (2 weight-training sessions a week as well as 3 sessions of aerobic activity). The peak oxygen consumption was 65% to 85% (starting the training course at 40% of peak oxygen consumption). We used heart-rate monitors (Bowflex, Nautilus Inc., Canada) for measurement of exercise-induced heart rates [220 – age × (65% to 85%)]. In addition, indirect calorimetry (Fitmate, Cosmed, Italy) was used to measure the exercise duration necessary to expend 10% of the daily caloric needs in each session for each individual.

At the start of the experiment, the participants had a weekly meeting. They learned how to stick to their dietary plans to increase their motivation and commitment to the program. Variations in activity levels or diets are associated with potential confounding effects during the course of such studies. Therefore, we recommended that they preserve their current PALs and diets throughout the study.

| Table 2. Subjects’ morphological characteristics in each group before intervention. |
|---------------------------------|---------------------------------|
| Group I (n = 15)               | Group II (n = 15)               |
| Sex, women/men, n              | 0/15                            | 0/15                            |
| Race, white/nonwhite, n        | 13/2                            | 12/3                            |
| Age, years                     | 59.2 ± 10.3                     | 58.4 ± 9.5                      |
| Height, cm                     | 174.4 ± 5.8                     | 173.7 ± 7                       |
| Systolic blood pressure, mean ± SD, mmHg | 129.1 ± 7.6                     | 130.6 ± 4.4                     |
| Diastolic blood pressure, mean ± SD, mmHg | 79.7 ± 2.1                     | 78.7 ± 2.6                      |
study. They were instructed to report any problems that could affect their involvement in the study.

Weight-loss assessment parameters and laboratory tests were performed before and after intervention for all subjects and were compared to each other. The method did not change during the study. We explained all procedures and requirements for the subjects. They voluntarily signed a consent form before enrolling in the study. The local ethics committee approved the study protocol.

All statistical data of the study were expressed as means ± standard deviations (SDs). The normal distribution of the collected data was evaluated using the Kolmogorov–Smirnov test. The data were normally distributed. The pre- and postintervention results for the groups were compared using the paired t-test, and the differences between the groups were evaluated by the independent t-test. Linear regression analysis (R) was used to examine a relation between all significant values (dependent) and weight change (independent). Statistical analysis was performed using SPSS 19.0 for Windows. P < 0.05 was considered statistically significant.

3. Results
Seven participants did not follow the study protocol, and thus they were excluded from further assessments. The final sample size was 30 men in good health (15 participants in each group). The morphological characteristics for individuals who completed the 3-month intervention are summarized in Table 2. The mean age and height of all participants (both groups) were 58.8 ± 9.77 years and 174.06 ± 6.35 cm. The mean age and height of individuals was not significantly different between groups. Most participants were white (approximately 83%), and the remaining were African (n = 3) and “other” (n = 2).

There were no significant differences in values of body weight, total C, HDL-C, and LDL-C in both groups before treatment. However, results of the assessments showed significant differences with respect to all the parameters of values between pre- and postintervention periods of both groups, as shown in Table 3.

There were no significant differences in body weight between NCD_sport (84.13 ± 10.39 kg) and LCD_sport (83.8 ± 9.62 kg) after intervention (P > 0.05). There was no significant difference with respect to total C and LDL-C values between groups after intervention (P > 0.05). Additionally, there were significant differences between the HDL-C levels of NCD_sport (54.86 ± 3.35 mg/dL) and LCD_sport (60.6 ± 4.15 mg/dL) (P < 0.001).

Linear regression analysis was implemented to find a relation between all significant values and weight change. This revealed a significant effect between weight change and HDL-C change. The regression coefficient was 0.2403 (P = 0.006) and 0.6057 (P < 0.0001) for NCD_sport and LCD_sport, respectively. Regression analysis confirmed that weight-loss predictions were more incremental in LCD_sport compared to NCD_sport (Figure). Analysis of the relationship between total C, LDL-C, and weight change revealed no significant effect (data not shown).

4. Discussion
We did not face trial limitations such as potential bias, multiplicity of analyses, etc. during the study, except for the lack of commitment of some participants to their individual PALs and/or diets; these participants were excluded from the study. Reasons for exclusion of subjects included changes in PAL and/or diet, drinking alcohol, or consuming sugar, honey, sugar substitutes, or any commercial dressings (high fat and sugar contents).

<table>
<thead>
<tr>
<th>Group I (NCD)</th>
<th>3 months, mean ± SD</th>
<th>Group II (LCD)</th>
<th>3 months, mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Weight, kg</strong></td>
<td>92.16 ± 10.37</td>
<td>84.13 ± 10.39*</td>
<td>92.36 ± 10.06</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>30.52 ± 3.33</td>
<td>27.93 ± 3.3</td>
<td>30.78 ± 2.93</td>
</tr>
<tr>
<td>Total C, mg/dL</td>
<td>192.2 ± 7.01</td>
<td>159.53 ± 7.01*</td>
<td>191.13 ± 4.77</td>
</tr>
<tr>
<td>HDL-C, mg/dL</td>
<td>51.53 ± 2.74</td>
<td>54.86 ± 3.35***</td>
<td>51.4 ± 3.5</td>
</tr>
<tr>
<td>LDL-C, mg/dL</td>
<td>124.13 ± 4.53</td>
<td>100.33 ± 4.99*</td>
<td>123.73 ± 4.31</td>
</tr>
<tr>
<td>Total C/HDL-C ratio</td>
<td>3.72 ± 0.29</td>
<td>2.92 ± 0.27***</td>
<td>3.69 ± 0.3</td>
</tr>
</tbody>
</table>

*: P < 0.05 compared to preintervention measurement.
***: P < 0.001 compared to Group II (LCD).
throughout the intervention. Aging is associated with degeneration, loss of functional ability, and obesity (18). Although age-related changes have a strong genetic component, they are also influenced by diet and physical activity. Thus, middle-aged and older men were chosen for the study. Weight loss and lipid profile changes with exercise and diet take at least 12 weeks and many previous investigators have used that timeframe (19,20). Therefore, the study ended after 3 months. Blood levels of TG are related to eating, and LCDs reduce these levels. Therefore, we removed the TG variable from this study.

All parameters, including weight, total C, HDL-C, and LDL-C, changed after intervention compared to before intervention in both groups, which demonstrates the effectiveness of both treatments. Both diets are low in fat. Accordingly, it seems that the cholesterol level in each diet had an important influence on the results. Our findings are consistent with the findings of Franz et al's study evaluating the weight-loss efficacy of dietary interventions, exercise, and meal replacements (21).

High blood lipids are risk factors for CVD that get worse with age (22,23). Reduced levels of weight, total C, and LDL-C induced by the NCD were similar to LCD results. Some other researchers have reported similar results for other diets (24,25).

HDL-C is the most important determining factor of CVD. Its change is critical in healthcare because one benchmark to estimate the risk of CVD is the ratio of total C to HDL-C (26–28). Our data showed that increment HDL-C levels in NCD_sport were significantly lower than in LCD_sport. It is known that exercise by itself causes HDL-C levels to increase. Our study has shown that exercise-induced increase in HDL-C levels is influenced by diet. This finding was confirmed in previous studies by other investigators (29). As shown in Table 3, the total C/HDL-C ratio changed in both experimental groups; there were significant differences in total C/HDL-C ratios between pre- and postintervention parameters. Decline in total C/HDL-C ratio was greater in LCD_sport than in NCD_sport. This finding may have clinical applications for weight loss with exercise.

Furthermore, results of the simple linear regression showed that HDL-C increased more in the LCD exercise group as compared to the NCD exercise group per weight unit increment (Figure).

Finally, it should be noted that fat is involved in building the membranes of all body cells. In addition, it provides essential fatty acids and vitamins A, D, and E. Fat in a NCD is less than the amount recommended by food standards. Because of their high carbohydrate content, such diets can contain over twice (40–70 g/day) the recommended amount of fiber (13). High fiber intake can decrease absorption of zinc, calcium, and iron (30). Complaints of flatus and abdominal fullness have also been reported (13). Therefore, it appears that this diet is unsuitable for long-term use and will probably lead to some problems.

In spite of many advertisements regarding NCDs, this diet has no different effect on weight loss than a LCD does. It seems that the concept of negative-calorie food has no external meaning or application. In addition, although some diets may have some effect on weight loss in the short-term, they can lead to remarkable side effects for the physical health of patients.

In conclusion, in contrast to the growing body of advertisements and their claims, both experimental groups showed similar patterns of weight loss. Under exercise conditions, weight loss obtained by a NCD had no advantage over LCDs regarding weight loss or the occurrence or development of cardiovascular dysfunctions.

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References


