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Effects of Mediterranean diet and weight loss on blood-lipid profile in overweight adults with hypercholesterolemia

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Summary. Blood cholesterol has been positively associated with increased cardiovascular risk as a modifiable risk factors together with the lifestyle and diet. Furthermore, an improvement of the blood-lipid profile seems to be able to produce a decrease in cardiovascular events. Cholesterol plasma levels are related to the body mass index (BMI) and are affected by diet. The aim of this study was to evaluate the effectiveness of a Mediterranean diet (MD) weight-loss programme to improve blood cholesterol profiles in overweight adults subjected to real-world outpatient diet. Forty-nine hypercholesteraemic, overweight adults of both sexes were subjected to a dietary weight-loss intervention. Patients were prescribed a slightly hypocaloric MD for 16 weeks, followed by an 8-week follow-up period with a normocaloric diet. Data showed significant weight loss and cholesterol blood profile improvement both under the hypocaloric diet and during the follow-up period. In particular, the decrease in both Total and LDL-cholesterol was greater than their critical differences indicating the clinical relevance of blood lipid improvement induced by MD.

Keywords: Cholesterol; Critical Difference; Mediterranean Adequacy Index; Nutritional Counselling; Triglycerides; Lifestyle.

Introduction

The blood-lipid profile has been positively associated with an increase in cardiovascular risk (CVR) by a large number of epidemiological studies. In particular, the reduction of total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) plasma levels induces a decrease in the incidence of cardiovascular events (1,2). By contrast, high-density lipoprotein cholesterol (HDL-C) is inversely related to CVR (3). An increase in TC -particularly in the LDL-C concentration - is a marker of atherogenic CVR, whereas a decrease in HDL-C concentration is correlated with various risk factors, including the development of metabolic syndrome (i.e., MetS) (4,5). Starting from this assumption, the TC/HDL-C ratio (well-known as "*Castelli atherogenic index*") seems to be a strong predictor of coronary heart disease and has got a high discriminatory capacity. For primary prevention, the TC/HDL-C ratio risk-thresholds have been set at 5 for males and 4.5 for females, with desirable targets of < 4.5 and < 4, respectively (5-7). European guidelines (2) focus on the reduction of LDL-C and TC blood concentrations as the main target in primary cardiovascular disease prevention. Furthermore, it has been suggested that pharmacological interventions (i.e., Statins) should be administered when the LDL-C concentration is greater than 190 mg/dL to achieve a desirable outcome of 155 mg/dL in patients with low CVR. Additionally, in patients with moderate CVR, the goals are to achieve a treatment threshold of 155 mg/dL with a desirable level of less than 120 mg/dL. In subjects with low-to-moderate CVR, recommendations include maintaining a healthy TC blood concentration less than 200

mg/dL, with borderline values considered between 200 mg/dL and 240 mg/dL.

In each person, cholesterol plasma levels are due to a balance between absorption after food-intake and endogenous synthesis (8,9). Therefore, genetic tendencies are influenced and modulated by metabolic factors, such as BMI (10) and the amount of visceral or liver fat (11). Furthermore, diet composition affects various plasma cholesterol concentrations and ratios (12,13). In particular, on one hand a reduction in the dietary intake of saturated fat acids (SFAs) is strictly related to both improvement of the blood-lipid profile (14,15) and lowering of CVR (16-18). On the other hand, epidemiological and clinical evidence is consistent with finding reporting a reduction of CVR depending on nutrients used to replace SFAs (15,19). Specifically, it has been demonstrated that the replacement of SFAs with unsaturated fatty acids, either monounsaturated fatty acids (MUFAs) or polyunsaturated fatty acids (PUFAs), can reduce CVR (17,20-22). Conversely, SFA-replacement with high-glycaemic-index (GI) refined carbohydrates actually increases CVR (23,24). Nevertheless, studies exploring the effects of nutritional interventions on lipid profiles never compared TC and/or LDL-C reduction with their critical differences (CDs) (12,14, 17, 25-28) CD, also known as the reference change value, is a parameter used to assess laboratory results (29,30). The CD is defined as the smallest difference between sequential laboratory results which is likely to indicate a true change for a given analyte in the patient. Particularly, the difference between two consecutive analyses is significant and clinically relevant if the difference between the two measurements is higher than the CD (30, 31).

Many studies provide direct evidence that a Mediterranean diet (MD), characterized by a high percentage of unsaturated fatty acids, especially MUFAs, and a high proportion of low-GI whole grains, is inversely associated with CVR(32-35) as well as MetS development (36, 37). However, in the Mediterranean region in recent years, a Westernization of dietary habits occurred with a corresponding shift away from the traditional Mediterranean nutritional (38, 39, 40).

Thus, the aim of this study was to assess the efficacy of a weight-loss programme based on the characteristics of an MD under real-world outpatient conditions to improve the blood-lipid profile in overweight patients with moderate to high hypercholesterolemia.

Materials and Methods

Participants

Fifty-five overweight adults with hypercholesterolemia, including 31 males and 24 females aged between 31 and 65 years (49 ± 7.4 years SD), were consecutively recruited from a larger sample of overweight adults subjected to a dietary weight-loss intervention. All participants had a sedentary job and did not engage in sufficient physical activity according to the recommendations of the World Health Organization (i.e., less than 150 minutes of moderate-intensity activity each week). The Ethics Committee for clinical trials of the University of Pisa (CEAVNO) approved the study protocol (nr. #271/2014). All participants provided written informed consent and data were processed anonymously by assigning an identification number to each patient. Six subjects (3 males and 3 females) withdrew before the end of the protocol, leaving 21 females and 28 males to be included in the study. Patients who dropped out of the study underwent pharmacological therapy. Table I shows the baseline characteristics of the participants who completed the protocol.

The inclusion criteria comprised 25 < BMI <30, TC ≥ 250 mg/dL (to convert mg/dL into mmol/L, multiply by 0.0259), and TC/HDL-C >5. Exclusion criteria included triglycerides ≥ 220 mg/dL (to convert mg/dL into mmol/L, multiply by 0.011), currently taking lipid-lowering medications, a personal history of cardiovascular disease, cancer, hypertension (ie., blood pressure ≥ 140/90 mm Hg), and diabetes, renal or liver disease.

Experimental Protocol

A nutritional assessment and medical (i.e., baseline blood profile), anthropometric, and psychological examinations were performed for each patient. Height was measured without shoes to the nearest centimetre at baseline using a wall-mounted stadiometer. Body weight was measured without clothes to the nearest 0.5 kg. During the first visit, the Eating Habits Structured Interview (EHSI) was administered, too. The EHSI is

Table 1. Characteristics of Male and Female groups at baseline. For normally distributed data, the Mean and Standard Deviation are reported; the Median, 1st and 3rd quartiles are shown in non-Gaussian distributions. Significance of the baseline differences between the sexes was calculated by * ANOVA Mixed design and Post Hoc test, ° Mann-Whitney U and + Unpaired Student t-test.

		Females	Males	P between sexes
Normality	**Subjects N°**	21	28	
Yes	Age (years) +	49.5 ± 7.4	48.7 ± 7.5	NS
Yes	Height (cm) +	162.9 ± 5.5	176.5 ± 5.2	< 0.0001
Yes	Weight (kg) +	72.7 ± 5.8	86.2 ± 7.1	< 0.0001
Yes	BMI (kg/m^2) *	27.4 ± 1.5	27.7 ± 1.3	NS
No	MAI °	2.5 (2.1-4.0)	2.6 (2.4-3.1)	NS
Yes	TC (mg/dl) *	278 ± 17.5	279 ± 17.9	NS
Yes	LDL-C (mg/dl) *	192 ± 15.5	194 ± 16.2	NS
No	HDL-C (mg/dl) °	48 (44-50)	42 (40-45)	< 0.001
No	TG (mg/dl) °	213 (199-215)	214 (205-216)	NS
Yes	TC/HDL-C *	5.9 ± 0.61	6.6 ± 0.63	< 0.001

an interview on dietary habits and lifestyles covering 5 sheets (i.e., master data and medical history, lifestyle, physical activity, body perception, and eating habits) with 53 items (41). The eating habits sheet included a report of the diet composition pertaining the previous week. Dietary composition data were processed in order to evaluate the adherence to an MD model using the Mediterranean adequacy index (MAI) (42), as described below.

At baseline and after fasting for 12 hours, venous blood samples were drawn without stasis into evacuated glass tubes. TC and triglyceride (TG) concentrations were measured enzymatically, HDL-C was measured through precipitation (43) and LDL-C was measured using Friedewald's formula. Biological Variability (CVb), Analytical Variability (CVa) and Critical Difference (CD) for TC, LDL-C, HDL-C and TG are reported in Table II, according to the Tuscany quality assessment program for clinical biochemistry. (44, 45).

Table 2. Biological Variability (CVb), Analytical Variability (CVa) and Critical Difference (CD) for Total Cholesterol (TC), LDL-Cholesterol (LDL-C), HDL-Cholesterol (HDL-C) and Triglycerides (TG) according to the Tuscany quality assessment program for clinical chemistry (Barsotti, 1995; La Gioia, 2013).

Analytes	CVb (%)	CVa (%)	CD (%)
TC	6.1	2	17.8
LDL-C	7.6	5	25.2
HDL-C	7.4	5	24.7
TG	8.1	3.5	24.4

Immediately after the first medical assessment, each participant received a personalized dietary weight-loss programme. The hypocaloric eating plan was carried out for 16 weeks, and then it was followed by 8 weeks during which the hypocaloric diet was replaced with a normocaloric one. Nutritional counselling sessions were performed every two weeks in order to evaluate both diet adherence and progression in the alimentary education programme. Weight data were processed at baseline, 8 weeks, 16 weeks and 24 weeks. In the fourth week (during the weight-loss programme) and twentieth week (during the follow-up period), each subject compiled a weekly dietary report, and data were processed to evaluate the MAI. Blood tests control was performed at 8 weeks, 16 weeks and 24 weeks. At the end of the protocol, the participants who did not meet the threshold values for the relative class of risk, underwent pharmacologic care, according to the ESC/EAS guidelines for the management of dyslipidaemia (2).

For each analyte, the differences between the end and the beginning of the protocol were calculated and reported as a percentage of the initial value. This percentage was compared to the relative CD and the number of subjects reporting a reduction in blood levels higher than the CD was reported.

Mediterranean Adequacy Index (MAI)

According to the Fidanza's formula, MAI was computed by dividing the sum of the percentages of

dietary energy (from food groups typical of a reference MD) by the sum of the percentages of dietary energy of food groups, that are not characteristic of the referent MD (42).

$$MAI = \frac{\%.energy\ .from.Mediterranean.foods}{\%.energy\ .from\ .non.Me\ diterranean.\ foods}$$

Foods typical of an MD were cereals and their derivatives, legumes, potatoes, vegetables, fresh and dry fruit, fish, wine, and extra-virgin olive oil. In the group of non-MD foods were milk and dairy products (including cheese), meat, eggs, animal fats and margarines, sweet beverages, cakes, pies, and cookies. The choice of the two food groups was based on the healthy MD proposed by Alberti and colleagues following the results of a dietary survey carried out in a sub-sample of Nicoteran (Southern Italy) families in 1960 (42, 46). For each subject, MAI was computed by calculating the weekly energy intake for each food group and relating it to the total energy intake, followed by an application of the Fidanza's formula.

Clinical Psychological Investigation

All subjects underwent a clinical psychological assessment, during which the Pisa Survey for Eating Disorders (PSED) was completed. The PSED is a self-administrated questionnaire for the determination of eating habits and self image (47) Additionally, all subjects underwent continuous clinical psychophysiological registration (PPR) of specific parameters strictly connected with the Autonomous Nervous System (ANS) arousal, in order to evaluate the balance between sympathetic and parasympathetic balance as previously described (48). The multichannel SATEM ("Modulab® 800") was used to carry out the PPR connected to a computer via an infrared cable, and data were detected and processed by the PANDA Works programme® software (SATEM). The purpose of the evaluation was to identify subjects with potentially full-blown eating disorders described above as a particular pattern of the ANS activity (49).

Diet Protocol

The resting energy expenditure was estimated using the Mifflin's formula(50) and adjusted for physical activity levels. Hypocaloric diet was based on total daily energy expenditure (TDEE) levels, removing approximately 25% of kilocalories (26.9% of the TDEE ± 0.8% SD) to achieve a 10% weight loss in a 16-week period for each participant. Eating plans for each subject were based on the MD model, by maintaining a protein intake of approximately 0.8 g/kg (0.83 ± 0.05 SD), as recommended by the Italian guidelines of the National Institute for Research on Food and Nutrition (INRAN) (51).

The daily macronutrient distribution was 52.3% (± 1.9 SD) carbohydrates, 18.7% (± 1.1 SD) proteins, and 29% (± 1.5 SD) fats with approximately 75% UFAs. Approximately 75-80% of the fats in the diet were of vegetable origin, and an MUFA: PUFA: SFA ratio of approximately 2:1:1 was maintained. The daily vegetable fibre intake was 29.5 g (± 2.05 SD). Nutritional characteristics of the foods used in the eating plans derived from the database of food composition published by the Italian Research Institute for Food and Nutrition (52).

Daily caloric contribution was distributed in five daily meals: breakfast (approximately 20% of the total daily caloric intake), morning snack (approximately 5% of the total daily caloric intake), lunch (approximately 35% of the total daily caloric intake), afternoon snack (approximately 10% of the total daily caloric intake), and dinner (approximately 30% of the total daily caloric intake). The daily diet included two servings of mixed vegetables (i.e., approximately 400 g), three servings of fruit (i.e., approximately 450 g), and one serving of semi-skimmed milk or yogurt (i.e., 125 g for serving). There were additional limitations on beef (i.e., once every ten days), pork (i.e., once per week), cheese (i.e., no more than once per week) and eggs (i.e., twice per week). These proteins were replaced with fish, poultry, rabbit and legumes. Complex carbohydrates were provided by pasta, spelt, brown rice, mixed wholegrain and whole-meal bread, while limiting the use of potatoes, rice and white bread. The GI of each meal was always maintained at less than 55%, as well as the glycaemic load (GL) was always less than 50. GI and GL were calculated as indicated by Wolever et al (53). Butter usage was replaced with 20-35 g/day of extra-virgin olive oil, depending on energy intake (i.e, approximately 15% of the total daily caloric intake).

Moreover, a diet integration of 10–15 g/day of nuts (i.e., approximately 6% of the total daily caloric intake) was provided. A daily consumption of 100 ml red wine was also granted. The same directions were applied in the follow-up diet without caloric restriction.

Statistical Analysis

Correlations at baseline between MAI and values of BMI, TC, LDL-C, HDL-C and TG were performed by non-parametric Spearman rs. Significance of correlation coefficients were tested by Fisher's formula.

The normal distribution of the samples and the homoscedasticity of their variances were evaluated with Shapiro-Wilk and Levene's tests, respectively. In normally distributed data (i.e., BMI, TC, LDL-C and TC/HDL-C), statistical evaluation was performed using a between-within Analysis of Variance in a within-between mixed design (sex - time (0, 8, 16 and 24 weeks)). A post hoc multiple comparison analysis of between data was performed, by the Tukey-Kramer HSD Test. For within data, paired Student t-tests with Bonferroni's correction were used. The effect size for a mixed between-within ANOVA was calculated by using the partial eta squared ($P\eta 2$). In non-parametric data (i.e., MAI, HDL-C and TG), within effects were analysed using Friedman's ANOVA. A post hoc multiple comparison analysis was performed by the Wilcoxon tests (Bonferroni corrected). Differences in gender were evaluated using multiple Mann-Whitney U tests (Bonferroni corrected). The effect size for non-parametric tests was calculated through an application of the non-parametric *r* to post hoc analysis.

To assess variations in the effects over time, we calculated differences between the eighth week and baseline ($\Delta 1$), the sixteenth and eighth weeks ($\Delta 2$), and the twenty-fourth and sixteenth weeks ($\Delta 3$) for all variables that changed during the protocol. For each analyte (i.e., TC, LDL-C, HDL-C and TG), the blood-levels variation (Δ_{Total}) between the end of the protocol (at the twenty-fourth week) and baseline was compared with related CDs. The correlation between the baseline values of TC, LDL-C, and TG and the corresponding Δ_{Total} was evaluated by Pearson ϱ in normally distributed data, and by Spearman rs in non-Gaussian data distributions. Significance of correlation coefficients were tested by Fisher's formula.

Results

Primary analyses

The overall adherence to the protocol was quite good, with a drop-out rate of 10.9% (6 subject out of 55). Adherence to the MD in the week before the start of the dietary programme was low (i.e., MAI Median = 2.61, 1st quartile = 2.32; 3rd quartile 3.48), but a highly significant reverse correlation between MAI and both TC and LDL-C concentrations at baseline was detected (rs = -0.57, $F_{(1,47)} = 22.86$, $P < 0.0001$ and rs = -0.52, $F_{(1,47)} = 17.33$, $P < 0.001$, respectively). No significant correlation between MAI and BMI, HDL-C or TG was found, too.

All the subjects showed a significant change of their eating habits, increasing adhesion to the Mediterranean diet. Indeed, statistical analysis showed a significant MAI increment over time (Friedman $\chi^2 = 74.5$, df = 2, $P < 0.0001$). The MAIs calculated during the weight-loss programme (at the fourth week) were significantly higher than those at baseline (Median = 5.11, 1st quartile = 5.0; 3rd quartile = 5.26; Wilcoxon $T_{(49/49)} = 0$, $p < 0.0001$, r = 0.87). These values were similar to the MAIs calculated in the twentieth week, during the follow-up period (Median = 5.15, 1st quartile = 5.04; 3rd quartile = 5.30; Wilcoxon $T_{(49/49)}=0$, $p < 0.0001$, r = 0.87), and no differences were found between the 4th and 20th weeks (Wilcoxon $T_{(49/49)} = 526$, $p > 0.25$, r = 0.05). We did not find any difference in eating habits between males and females ($p > 0.1$).

Weight loss

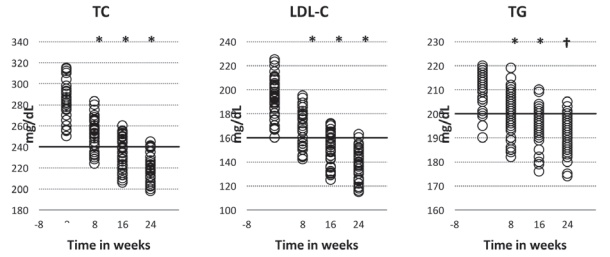
During the dietary weight-loss intervention (baseline-sixteenth week), all the subjects showed a constant reduction in BMI. No sex-related differences were detected in BMI reduction (ANOVA, between subjects $p > 0.5$; $F_{(1,47)} = 0.01$, $P\eta^2 = 0.000$), whereas a high statistical significance was found in diet-treatment (ANOVA, within subjects $p < 0.0001$; $F_{(3,141)} = 1468$; $P\eta^2 = 0.97$). Post hoc analysis showed an awesome decrease in BMI after 8 and 16 weeks of the hypocaloric diet (Bonferroni's paired Student t-test, $p < 0.0001$). However, no differences were observed between the sixteenth and twentieth week after the normocaloric diet period (Bonferroni's paired Student t-test, $p > 0.1$). The mean difference between baseline values and those collected at the sixteenth week was 2.67 ± 0.34 SD, approximately

10% of the baseline BMI (9.6% ± 1.27% SD). Weight loss was gradual, and no differences between D_1 (1.4 ± 0.26) and D_2 (1.3 ± 0.27) were found (Wilcoxon $T_{(49/49)}$ = 310.5, p > 0.5, r = 0.09). No correlation between baseline BMI and the TC, LDL-C, HDL-C, and TG concentrations were found.

Total Cholesterol (TC) and LDL-Cholesterol (LDL-C)

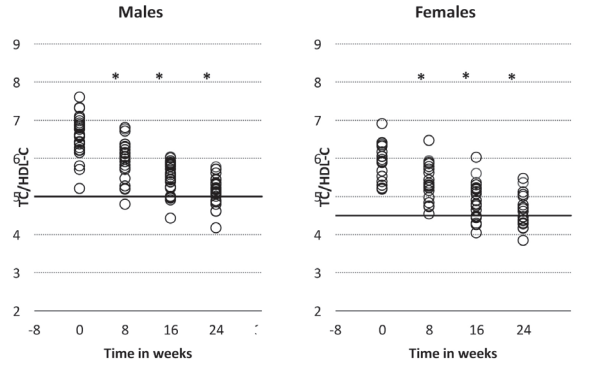
TC and LDL-C showed similar patterns of variation. No differences or interactions in sex were found in blood concentrations. Conversely, all the subjects manifested a consistent and significant reduction of both analytes concentration during the study protocol (Figure 1). Post hoc analysis showed significant differences among all the measurements as well as between the end of the weight-loss programme (sixteenth week) and the end of the normocaloric diet follow-up (twenty-fourth week) (Figure 1). Nevertheless, the decrease in TC and LDL-C slowed down over time. In fact, there were significant differences among Δ_1, Δ_2 and Δ_3, and post hoc analysis detected that $\Delta_3 < \Delta_2 < \Delta_1$ (Figure 2). The decrease during the follow-up period (Δ_3) was approximately 20% of the D_{Total} for both TC and LDL-C (19.2% ± 3.5% SD and 20.2% ± 3.9% SD, respectively). Furthermore, both TC and LDL-C showed a strong correlation between baseline values and D_{Total} (ϱ = -0.75, $F_{(1,47)}$ = 55.77, p < 0.0001 and ϱ = -0.71, $F_{(1,47)}$ = 43,9.77, p < 0.0001, respectively). At the end of the protocol, 94% of the subjects (n = 52) showed a reduction of TC greater than the CD. Similarly, 86% of the subjects (n = 49) showed a reduction of LDL-C higher than the CD (Table III).

Although 92% of the subjects (26 males (93%) and 19 Females (90%)) showed a TC concentration less than 240 mg/dat at the end of the protocol, only



		baseline	Weight loss 8th week	Weight loss 16th week	Weight loss 24th week	Follow up	Within Subjects (Time)
TC (mg/dl)	Males	280 ±18.0 SD	252 ±15.9 SD	234 ±15.0 SD	223 ±14.0 SD		**P <0.0001 ANOVA** $F_{(3,141)}$ = 3049.34 $Pη^2$ = 0.98
	Females	278±18.5 SD	253±16.1 SD	234±14.5 SD	223±12.8 SD		
	Between Subjects (Sex) ANOVA	**P >0.5**; $F_{(1,47)}$ = 0.002, $Pη^2$ = 0.001					**Interaction (Sex x Time) P >0.5** $F_{(3,141)}$ = 0.69 $Pη^2$ = 0.01
LDL-C (mg/dl)	Males	194±16 SD	164±14 SD	141±13 SD	133±12 SD		**P <0.0001 ANOVA** $F_{(3,141)}$ = 1468.06 $Pη^2$ = 0.97
	Females	194±16 SD	165±14 SD	142±14 SD	135±13 SD		
	Between Subjects (Sex) ANOVA	**P >0.5**; $F_{(1,47)}$ = 0.27, $Pη^2$ = 0.006					**Interaction (Sex x Time) P >0.5** $F_{(3,141)}$ = 0.269 $Pη^2$ = 0.006
TG (mg/dl)	Males	213(208-216)	201(197-205)	196(193-199)	195(191-197)		**P <0.0001 Friedman** c^2 = 120 df = 3
	Females	213(210-215)	203(195-207)	195(191-199)	192(185-196)		
	Between Subjects (Sex) Mann W U	**P>0.25**	**P>0.5**	**P>0.25**	**P>0.1**		

Figure 1. Changes in Total Cholesterol (TC), Low-Density Lipoprotein-Cholesterol (LDL-C) and Triglycerides (TG) during the treatment of males and females. Within post hoc comparison significances: * P<0.0001 and † P<0.001. Paired Student t-test with Bonferroni's correction in normally distributed data and Wilcoxon Matched Pairs Test with Bonferroni's correction in non-parametric analyses. In the table, the mean ± standard deviation (SD) is reported for each time point, and the results of the split-plot (between-within mixed design) ANOVA for TC and LDL-C are reported. For TG, the median and 1st and 3rd Quartiles are shown, and non–parametric statistical analyses are reported (Friedman for within data and Mann Whitney U with Bonferroni's correction for multiple between-data comparisons).



	baseline	Weight loss 8th week	Weight loss 16th week	Follow up 24th week	ANOVA Within Subjects (Time)
Males	6.58±0.51 SD	5.98±0.48 SD	5.47±0.38 SD	5.20±0.38 SD	**P <0.0001** $F_{(3,141)}$ = 741.3 $Pη^2$ = 0.94
Females	5.86±0.46 SD	5.35±0.49 SD	4.89±0.48 SD	4.66±0.43 SD	
ANOVA Between Subjects (Sex)	**P <0.0001**; $F_{(1,47)}$ = 23.02, $Pη^2$ = 0.33				**Interaction (Sex x Time) P <0.01** $F_{(3,141)}$ = 4.50 $Pη^2$ = 0.09
Post Hoc between (Sex) Tukey-Kramer	P <0.001	P <0.01	P <0.01	P <0.01	

Figure 2. Changes in Total Cholesterol (TC), Low-Density Lipoprotein-Cholesterol (LDL-C) and Triglycerides (TG) during the treatment of males and females. Within post hoc comparison significances: * P<0.0001 and † P<0.001. Paired Student t-test with Bonferroni's correction in normally distributed data and Wilcoxon Matched Pairs Test with Bonferroni's correction in non-parametric analyses. In the table, the mean ± standard deviation (SD) is reported for each time point, and the results of the split-plot (between-within mixed design) ANOVA for TC and LDL-C are reported. For TG, the median and 1st and 3rd Quartiles are shown, and non–parametric statistical analyses are reported (Friedman for within data and Mann Whitney U with Bonferroni's correction for multiple between-data comparisons).

Table 3. Mean and Standard Deviation of the total variation (Δ_{Total}) of each analysis at the end of protocol (24th week) compared with the Critical Difference (CD) according to the Tuscany quality assessment program for clinical chemistry (22). N Δ_{Total} > CD Numbers of subjects showing a reduction higher than the Critical Difference.

		Δ_{Total} (mg/dl)	Δ_{Total} (%)	CD (%)	N Δ_{Total}>CD	N Subjects
TC (mg/dl)	M	-63.7±8.7 SD	22.7±2.3 SD	17.8	27	28
	F	-62.6±9.6 SD	22.3±2.4 SD		19	21
LDL-C (mg/dl)	M	-60.5±9.2 SD	30.9±3.5 SD	25.2	25	28
	F	-60.3±9.0 SD	31.3±3.2 SD		17	21
TG (mg/dl)	M	-17.7±11.2 SD	8.2±5.2 SD	24.4	0	28
	F	-12.6±8.7 SD	6.2±4.2 SD		0	21

4% of them (2 males (7%)) reached a TC concentration less than 200 mg/dl. By contrast, all the subjects showed an LDL-C concentration lower than 190 mg/dl, and 86% of participants (20 females (95%) and 22 males (79%)) reached a concentration less than 155 mg/dl. Furthermore, in 3 males (11%) and 2 females (10%), LDL-C decreased under 120 mg/dl.

Triglycerides (TGs)

TG plasma concentrations showed a pattern of variation similar to that of TC and LDL-C with no significant differences between sexes and a statistically significant decrease of the analyte concentration during treatment. Post hoc analysis showed significant variations between the eighth week and baseline, between the sixteenth and eighth weeks, and between the twenty-fourth and sixteenth weeks (Figure 1). Similarly to the decrease in TC and LDL-C, TG decrease slowed down over time, particularly $\Delta_3 < \Delta_2 < \Delta_1$ (Figure 2). Moreover, TG showed a correlation between baseline values and D_{Total} (ϱ = -0.65, $F_{(1,47)}$ = 34.4, p < 0.0001). The decrease during the non-energy-restricted diet follow-up period was less than that of TC and LDL-C (10.7% ± 7.5% SD). Despite the significant TG blood-concentration decrease during treatment, no subjects showed a reduction of TG greater than the CD at the end of the protocol (Table III).

HDL-Cholesterol (HDL-C)

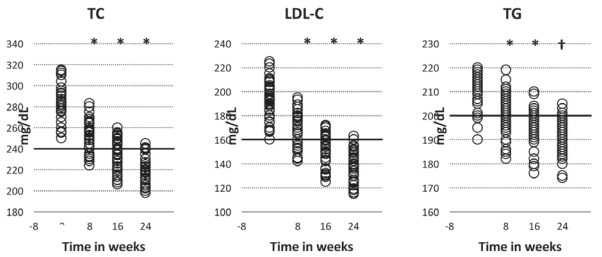
Significant differences between sexes in HDL-C blood concentrations were found, with females showing higher values than males in every instance (p < 0.005, Mann-Whitney U). In contrast, significant effects of diet were not found in males or females (females, Friedman: c^2 = 1.49, df = 3, p > 0.5; males, Friedman: c^2 = 5.99, df = 3, p > 0.1).

TC/HDL-C

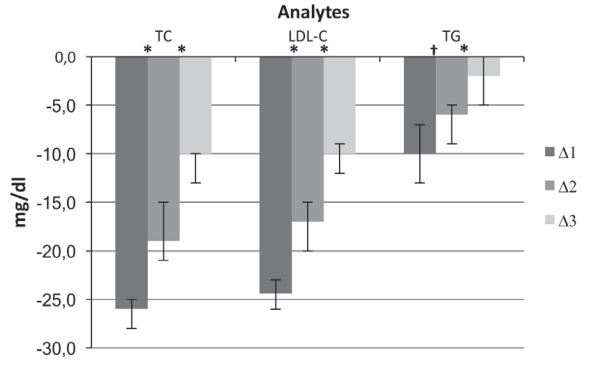
A significant difference between sexes was found in the TC/HDL-C ratio, with males manifesting consistently higher values. Both males and females showed a highly significant reduction of the TC/HDL-C ratio over the study period, and post hoc analysis indicated significant differences during all treatment periods (Figure 3). In addition, a significant interaction between sex and diet effects was found (Figure 3). In fact, the reduction in the TC/HDL-C ratio in males (DTotal = -1.38 ± 0.26 SD) was significantly higher than in females (DTotal = -1.22 ± 0.21 SD; Student t-test, p < 0.05) at the end of the protocol. At the end of the treatment, 42.9% of females (n. = 9) reached a TC/HDL-C ratio less than 4.5, and 21.4% of males (n. = 8) had a TC/HDL-C ratio less than 5.

Discussion

To the best of our knowledge, the results of this study show for the first time a reduction in TC and LDL-C blood concentrations achieved by a lifestyle intervention greater than the CD. Previous studies exploring the effects of weight loss, nutritional interventions, and/or both on lipid profiles has never compared TC and/or LDL-C reduction with CD (12,14, 17, 25-28). We found a clinically relevant reduction in TC and LDL-C without a decrease in HDL-C induced by both MD and weight-loss. These findings are supported by the significant decrease in Castelli's index for each participant. The decrease in TG - although statistically significant - never reached a rate of decline that was higher than the CD. Differences found between males and females in HDL-C concentration and in TC/HDL-C ratio are consistent with previously pub-



	baseline	Weight loss			Follow up	Within Subjects (Time)
		8th week	16th week	24th week		
TC (mg/dl)	Males	280±18.0 SD	252±15.9 SD	234±15.0 SD	223±14.0 SD	**P <0.0001** ANOVA $F_{(3,141)} = 3049.34$ $Pη^2 = 0.98$
	Females	278±18.5 SD	253±16.1 SD	234±14.5 SD	223±12.8 SD	
	Between Subjects (Sex) ANOVA	**P >0.5**; $F_{(1,47)} = 0.002$, $Pη^2 = 0.001$				**Interaction (Sex x Time) P >0.5** $F_{(3,141)} = 0.69$ $Pη^2 = 0.01$
LDL-C (mg/dl)	Males	194±16 SD	164±14 SD	141±13 SD	133±12 SD	**P <0.0001** ANOVA $F_{(3,141)} = 1468.06$ $Pη^2 = 0.97$
	Females	194±16 SD	165±14 SD	142±14 SD	135±13 SD	
	Between Subjects (Sex) ANOVA	**P >0.5**; $F_{(1,47)} = 0.27$, $Pη^2 = 0.006$				**Interaction (Sex x Time) P >0.5** $F_{(3,141)} = 0.269$ $Pη^2 = 0.006$
TG (mg/dl)	Males	213(208-216)	201(197-205)	196(193-199)	195(191-197)	**P <0.0001 Friedman** $\chi^2 = 120$ df = 3
	Females	213(210-215)	203(195-207)	195(191-199)	192(185-196)	
	Between Subjects (Sex) Mann W U	**P>0.25**	**P>0.5**	**P>0.25**	**P>0.1**	



	Weight loss			Follow up	ANOVA
	baseline	8th week	16th week	24th week	Within Subjects (Time)
Males	6.58±0.51 SD	5.98±0.48 SD	5.47±0.38 SD	5.20±0.38 SD	**P <0.0001** $F_{(3,141)} = 741.3$ $Pη^2 = 0.94$
Females	5.86±0.46 SD	5.35±0.49 SD	4.89±0.48 SD	4.66±0.43 SD	
ANOVA Between Subjects (Sex)	**P <0.0001**; $F_{(1,47)} = 23.02$, $Pη^2 = 0.33$				**Interaction (Sex x Time) P <0.01** $F_{(3,141)} = 4.50$ $Pη^2 = 0.09$
Post Hoc between (Sex) Tukey-Kramer	P <0.001	P <0.01	P <0.01	P <0.01	

Figure 3: Changes in Castelli's index (TC/HDL-C) during the treatment of both males and females. * indicates the within-data post hoc comparison significances (P< 0.0001 paired Student t-tests with Bonferroni's correction). The solid lines represent the risk-threshold: 5 for males and 4.5 for females. In the table, the mean ± standard deviation (SD) is reported at each time-point, including the results of the split-plot (between-within mixed design) ANOVA.

lished data (53, 54).

The changes that occurred during the hypocaloric diet period are clearly due to both the weight loss and the nutritional profile and TC, LDL-C and TC/HDL-C also decreased during the follow-up period without any caloric restriction, demonstrating a direct effect of the MD nutritional pattern in improving cholesterol profile. The magnitude of the changes during the normocaloric period was lower than in the first periods of the weight-loss programme, and this is partially due to the direct effect of negative energetic balance during the hypocaloric diet (56). However, we found a progressive decrease in the efficacy of the programme in improving the lipid-profile that could partially explain the lower rate of decline of TC, LDL-C and TC/HDL-C at the end of the protocol. The magnitude of changes in TG during the follow-up period of the normocaloric diet was less than that in TC and LDL-C. This could be partially explained by the progressive slowing of the TG decrease but it would seem to indicate that the decrease in TG was more closely related to weight loss than to nutritional pattern. Moreover, we found a statistically significant reverse-correlation between MAI scores and both TC and LDL-C concentrations before the start of the program, confirming data previously reported by Platania et al (57). These findings seems to indicate a positive correlation between the adherence to MD and an healthier blood lipid profile.

Furthermore, our findings showed a key role of the MD on the control of the blood-lipid profile, particularly in lowering atherogenic TC and LDL-C without decreasing the healthy HDL-C fraction. Despite traditional recommendations suggesting that low-cholesterol and low-fat diets may improve dyslipidaemia and metabolic disorders, the typical fatty acid profile and carbohydrate sources rich in fibres, folates and phytosterols (i.e., fruits, vegetables, and whole grains) that characterize the MD have been demonstrated to be more effective than interventions based on the limitation of individual food categories alone (39). These features of the MD are likely partially responsible for the protective effect of this diet against CV disease and follow the European guidelines for CV prevention(2).

Our data further showed a low baseline adherence to the Mediterranean dietary pattern, confirming previous indicating a clear tendency towards Westernization of eating habits (38-40). Nevertheless, temporal variations in the MAI that were induced by the dietary programme showed the possibility for improvement in dietary habits, accompanied by a positive progres-

sion of clinical parameters in the blood-lipid profile. Moreover, MD seems to have a good rate of compliance with drop-out rate of 10.9%.

Our study was a single-arm study, and the lack of a control group represents its principal limitation. However, our aim was to test the efficacy of a MD weight-loss programme to improve the blood-lipid profile under real-world outpatient conditions. Furthermore, the comparison of the CD demonstrates the clinical relevance of our findings and their applicability for outpatients.

The overall results of our study suggest that the Mediterranean Diet should be treated as a valuable supportive tool in controlling the serum blood-lipid profile, particularly if the MD is associated with a negative energetic balance. Indeed, the MD may be considered a healthy and sustainable lifestyle that plays a key role in both weight control and the primary prevention against CV disease.

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