

ORIGINAL ARTICLE

The Effect of Mediterranean Diet and High-Intensity Interval Training on Lipid Profile and HbA1c Level among Overweight and Obese Female Population

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ABSTRACT

Background: Dyslipidemia and impaired glucose tolerance are often associated with obesity and cardiovascular diseases. Based on beneficial effects of Mediterranean Diet (MD) and High-Intensity Interval Training (HIIT) on cardiovascular risk factors, the present study aimed to assess these effects on serum levels of total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and hemoglobin (Hb) A1C of overweight and obese female population.

Methods: Forty-seven participants, with an average age of 34.43 ± 7.22 years were assigned to four groups of MD, HIIT, MD+HIIT, and a control group. The interventions lasted 8 weeks, MD was without calorie restriction and HIIT exercise was on a cycle ergometer. Lipid profile of TC, TG, HDL-C, and LDL-C, and HbA1C were measured before and after all interventions.

Results: HbA1C exhibited a significant decrease in all intervention groups. A significant decrease in total cholesterol was observed in MD+HIIT group. Changes in TG, HDL-C, and LDL-C were not significant in any of the groups.

Conclusion: HbA1c was more responsive to MD and HIIT, while lipid profile exhibited greater resistance to any change. Despite non-significant modifications, MD+HIIT had the most favorable effects on LDL-C and TG levels.

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Introduction

Obesity is considered as the second leading cause of preventable deaths that plays a significant role and a risk factor for cardiovascular diseases (CVD) and comorbidities such as osteoarthritis and stroke (1). Among the prevalent aggressive

factors for endothelial tissue, obesity, dyslipidemia and impaired glucose tolerance play pivotal roles. Dyslipidemia that is characterized by an elevated triglyceride (TG), reduced high-density lipoprotein cholesterol (HDL-C), and an impaired glucose tolerance serve as a significant risk factor for CVD

and atherosclerosis (2). It was shown that levels of low-density lipoprotein cholesterol (LDL-C) and TG can be robust predictors for coronary heart disease (CHD) (3). Likewise, hemoglobin A1c (HbA1c) level that is a steady index of glycemic control can be an optimal marker of predicting microvascular complications (4).

Improvement in diet type and physical activity was shown to decrease the prevalence of obesity and reduce the risk of dyslipidemia and dysglycemia (5). Mediterranean Diet (MD) (6) originating from the pivotal Seven Countries Study (7) has been demonstrated to have favorable impacts on glycemic control and cardiovascular risk factors (8, 9). MD has been advocated for high consumption that can be found in plant-based foods, including fruits, vegetables, nuts, legumes, and whole grains; while the consumption of animal-source foods such as dairy, red meat, and processed meat is limited in MD. Additionally, MD covers the use of unsaturated fats, particularly extra virgin olive oil (10).

Likewise, regular physical activity by improving blood sugar and lipid profile can prevent progression of atherosclerosis (11). Among physical activities, high-intensity interval training (HIIT) is an emerging form of aerobic exercise that can condense exercise sessions more effectively in comparison to moderate-intensity continuous training (MICT) (2). HIIT is consisted of short bursts of intense exercise that is followed by active or passive rest intervals. It has the advantage of a vigorous exercise that can enhance various health-related factors, such as glycemic control (12) and lipid profile (13). The latest report from the American Diabetes Association has also advocated HIIT as an alternative to MICT in individuals with type 2 diabetes (14). Based on beneficial effects of MD (8, 15) and HIIT (16) on CVD and its risk factors, the present study compared the effect of MD and HIIT on serum levels of total cholesterol (TC), TG, HDL-C, LDL-C, and HbA1C.

Materials and Methods

The present controlled trial was approved by the Research Ethics Committee of Shiraz University (Approval ID: IR.US.REC.1401.009) and registered as IRCT20130424013107N4 in Shiraz University to investigate the effect of an 8-week MD and HIIT on serum lipid profile of TC, TG, HDL-C, and LDL-C, and HbA1C in overweight and obese female population between June and September 2022. All participants provided an informed consent before the enrollment. In our clinical trial, type one error (α) was 0.05, the power ($1-\beta$) was 80%, and the drop rate was 10% as described before (17). We estimated a minimum sample size of 45 and used

a convenience sampling method, while recruitment of samples took place in a diet therapy clinic in Shiraz, south of Iran.

A total of 47 women were enrolled in the study and were randomly allocated to 4 groups using block randomization method. Participants were enrolled if met the criteria of being 18-50 years, having a body mass index (BMI) more than 23 kg/m², being overweight or obese according to the BMI classification for the South Asian population (18). The exclusion criteria were not willing to participate in the training program for more than three sessions or failing to adhere to MD principles. Out of the 47 participants, 35 women were finally included in the study. Twelve participants were excluded from the study due to non-compliance with the proposed intervention program.

The four groups were defined as MD, HIIT, MD combined with HIIT (MD+HIIT) and control groups. The participants were instructed to adhere to MD without caloric restriction and assessed by Mediterranean Diet Serving Score (MDSS) with scores ranging from 0 to 24 for 14 dietary sections (19). MDSS evaluated the recommended consumption of foods and food categories per day and week. MD group maintained their usual physical activity habits too. MDSS overall score was calculated out of 23. It was defined as 3 points for fruits, vegetables, whole grains, and olive oil; 2 points for nuts and dairy products; and 1 point for legumes, fish, egg, poultry, red meat, sweets, and potatoes (alcoholic beverages were excluded). Fermented alcoholic beverages were not regularly consumed by any of the participants and were omitted from the analysis. Participants in the HIIT and HIIT+MD groups underwent supervised HIIT sessions at Shiraz University Gym. During an 8-week interval, 24 HIIT sessions were performed (3 sessions per week on nonconsecutive days). Each session lasted 20 minutes with a 5-minute warm-up at a self-selected speed, 4-6 bouts of 1-minute effort at 90-95% of a peak heart rate (HR-peak) interspersed with 1 minute of passive recovery, and a 3-minute cool-down. This HIIT protocol followed the recommendations from a previous study (20). Participants in the HIIT group were instructed to maintain their usual diet and nutritional habits too.

During the first visit, participants completed a 24-hour dietary recall and MDSS. About 3-7 days before the intervention, baseline assessments were undertaken including a comprehensive clinical evaluation by measurements of height (Seca 206 Roll-up measuring tape), body mass index (BMI), body composition (Inbody, model 230), waist and hip circumference. Blood samples were obtained for biochemical measurements. After 2 months,

participants who completed the study repeated these measurements. All assessments were conducted in the morning (before 10 am) and participants were fasted for a minimum of 8 hours prior to measurements and refrained from intentional physical activity on the morning of the examination. The measurements were carried out by the same examiner who was blind to the study.

The collected blood samples were kept in chilled heparinized tubes and were rapidly centrifuged and

stored until analysis. HbA1c was measured using Direct Enzymatic kit (ZiestChem Diagnostics), and lipid profile for TC, HDL-C, LDL-C, and TG were examined through Colorimetry method (Biorex Diagnostics). Per-protocol analyses (PPA) enrolled 35 adults who achieved at least 75% of MDSS or participated in 75% of the training sessions. SPSS Statistics software (Version 20, Chicago, IL, USA) and GraphPad Prism (Version 9.0.0 for Windows, GraphPad Software, San Diego, California, USA)

Table 1: Age, BMI, and serum levels of TC, TG, LDL, HDL, and HbA1c.

Variable	MD Mean (SD) (n=14)	MD+HIIT Mean (SD) (n=12)	HIIT Mean (SD) (n=12)	Control Mean (SD) (n=9)	Total Mean (SD) (n=47)	P value
Age (years)	34.43 (7.53)	35.42 (6.77)	34.83 (6.86)	37.78 (8.46)	35.43 (7.22)	0.742
BMI (kg/m ²)	26.77 (2.51)	26.78 (2.10)	26.46 (1.99)	26.75 (2.15)	26.69 (2.15)	0.981
Total Cholesterol (mg/dL)	179.71 (41.19)	175.33 (25.91)	191.58 (38.64)	192.33 (52.08)	184.04 (39.02)	0.668
TG (mg/dL)	98.00 (39.05)	97.00 (50.51)	90.08 (23.20)	121.55 (108.61)	100.23 (57.80)	0.661
LDL-C (mg/dL)	94.21 (26.64)	87.50 (20.66)	105.50 (34.50)	101.88 (43.96)	96.85 (31.19)	0.519
HDL-C (mg/dL)	47.78 (10.86)	50.25 (7.05)	49.66 (6.86)	51.55 (7.53)	49.61 (8.26)	0.753
HbA1c (%)	5.58 (0.21)	5.60 (0.25)	5.46 (0.47)	5.66 (0.19)	5.57 (0.30)	0.507

MD: Mediterranean diet, HIIT: High intensity interval training, BMI: Body mass index, TG: Triglyceride, LDL-C: Low density lipoprotein cholesterol, HDL-C: High density lipoprotein cholesterol, TC: total cholesterol, HbA1c: Hemoglobin A1c. Data were presented as mean±standard deviation (mean±SD). P values were presented from comparison between groups using ANOVA. Significance level was set at 0.05.

Table 2: Serum levels of TC, TG, LDL, HDL, and HbA1c before and after interventions.

Variable	Group	Pre-intervention Mean (SD)	Post-intervention Mean (SD)	Change%	t value	df	P value (t test)	P value (ANOVA)
Total cholesterol (mg/dL)	MD	186.37 (47.61)	194.75 (49.22)	+4.49	-0.789	7	0.456	0.124
	HIIT	185.66 (38.41)	186.00 (30.73)	+0.18	-0.068	8	0.948	
	MD+HIIT	176.80 (25.62)	165.30 (25.88)	-6.50	2.577	9	0.030	
	Control	177.75 (30.22)	183.50 (30.18)	+3.23	-1.468	7	0.186	
TG (mg/dL)	MD	107.12 (45.04)	113.12 (59.07)	+5.60	-0.311	7	0.765	0.478
	HIIT	85.88 (22.49)	107.11 (55.28)	+24.72	-1.323	8	0.222	
	MD+HIIT	97.80 (53.31)	90.80 (54.17)	-7.15	0.879	9	0.402	
	Control	86.12 (23.89)	93.62 (29.55)	+8.70	-1.791	7	0.116	
LDL-C (mg/dL)	MD	100.25 (32.81)	106.87 (38.72)	+6.60	-0.863	7	0.417	0.150
	HIIT	103.00 (34.46)	101.00 (26.80)	-1.94	0.54	8	0.604	
	MD+HIIT	84.80 (21.34)	78.70 (18.66)	-7.19	2.10	9	0.064	
	Control	89.12 (23.08)	96.37 (20.38)	+8.13	-1.56	7	0.162	
HDL-C (mg/dL)	MD	46.75 (8.10)	56.25 (6.49)	+20.32	-3.01	7	0.417	0.187
	HIIT	50.22 (6.94)	53.33 (9.15)	+6.19	-1.23	8	0.254	
	MD+HIIT	50.90 (7.07)	55.20 (11.18)	+8.44	-1.85	9	0.097	
	Control	52.12 (7.84)	53.87 (5.19)	+3.35	-0.95	7	0.371	
HbA1c (%)	MD	5.62 (0.23)	4.88 (0.70)	-13.16	3.64	7	0.008	0.007
	HIIT	5.56 (0.11)	4.88 (0.49)	-12.23	4.24	8	0.003	
	MD+HIIT	5.58 (0.27)	4.95 (0.41)	-11.29	6.09	9	0.000	
	Control	5.65 (0.20)	5.62 (0.34)	-0.53	0.25	7	0.805	

MD: Mediterranean diet, HIIT: High intensity interval training, TG: Triglyceride, LDL-C: Low density lipoprotein cholesterol, HDL-C: High density lipoprotein cholesterol, TC: total cholesterol, HbA1c: Hemoglobin A1c. df: degrees of freedom. Data were presented as the mean±standard deviation (mean±SD) for study groups. P-values, t, and df were presented from comparison of pre- and post-intervention measurements via paired t-test analysis. P values were also presented from comparison of mean changes between groups utilizing ANOVA. Significance level was set at 0.05.

were used for all analyses. Variables were presented as mean±standard deviation (SD). The t-test was employed to compare pre- and post- values of biochemical measurements within each group. One-way analysis of variance (ANOVA) analyzed the differences between the groups. In case of significant findings, the Tukey follow-up test was applied to compare paired groups. A p value<0.05 was considered statistically significant.

Results

Table 1 shows the characteristics of the 47 women including demographic, anthropometric, and biochemical variables of all groups. The mean±SD for age was 35.43±7.22 years, and HbA1c mean value was 5.57±0.30%. Table 2 outlines the changes after the intervention and the differences in mean changes between groups. ANOVA revealed that only the changes in HbA1c level were significant between the study groups ($p=0.007$). Subsequent Tukey follow-up tests (data not shown) indicated that differences between the control group and the other groups were statistically significant. Upon comparing pre- and post-intervention measurements, HbA1c significantly decreased in all intervention groups. TC exhibited a significant decrease only in the MD+HIIT group and changes in TG, HDL-C, and LDL-C were not significant in any of the groups.

Discussion

Obesity is considered as a risk factor for CVD and the associated comorbidities (21). Changes in diet such as utilizing MD (6) together with regular physical activity (11) can have cardiovascular benefits. Our findings revealed varying responses of glycemic and lipid markers to 8-week interventions of MD, HIIT, and MD+HIIT. The interventions showed the most favorable effect on HDL-C level as a crucial component of cardiovascular health. MD was demonstrated to improve lipid profile by increased consumption of polyunsaturated fatty acids (PUFA), dietary fiber, and fish oil, along with a decrease in consumption of saturated fatty acids (SFA) (3).

LDL-C was indicated to increase when SFA was replaced by carbohydrates; however, replacing carbohydrates with n-6 PUFA resulted in a lower LDL-C and a higher HDL-C level (22). Additionally, consuming N-3 PUFA can decrease TG level, while a higher sugar intake can significantly increase TG, TC, and LDL-C levels (3). As MD is rich in n-3 and n-6 PUFA, and low in SFA and sugar content, it can positively affect LDL-C, TG and HDL-C levels. Furthermore, it was shown that diet can rapidly

modify lipid profile, while 80% of the maximum effect happen within 2 weeks, and plateauing occur after 4 weeks (3).

Our findings revealed that MD could improve lipid profile. Similar to our results, a Cochrane review found minimal alterations following MD intervention in TC, LDL-C, and HDL-C levels (23). In the Women's Health Study (WHS) cohort, MD could decrease risk of diabetes by 30% (24) via a reduction in insulin resistance, inflammation, and inducing favorable effects on lipoprotein metabolism. Furthermore, among participants with higher MD scores, an increase in HDL-C level and a decrease in TG level were observed (24). In the WHS cohort study, only women with a baseline BMI of at least 25 demonstrated an inverse association between MD and type 2 diabetes (24). When the MD group was advised to consume olive oil, to increase the intake of nuts and seeds rich in n-6 PFA, to elevate the consumption of whole grains, and to include fish in their diet at least twice a week, MD could improve TC, TG, HDL-C, and LDL-C levels (25). Weight loss was also demonstrated to be correlated with changes in lipid profile that can explain differences in findings of different studies (3).

The HIIT group in our study demonstrated no significant change in lipid profile. TG level in the MD and HIIT groups were illustrated to increase by 5.60% and 24.72%, respectively; but not significantly. Conversely, in MD+HIIT group, a non-significant decrease of 7.15% in TG level was shown. Regarding LDL level, the MD group exhibited a non-significant increase of 6.60%, while the HIIT and MD+HIIT groups displayed a non-significant decrease of 1.94% and 7.19%, respectively. The combination of MD and HIIT could result in more favorable effects to improve TG and LDL levels. This aligns with a previous research that a 12-week HIIT intervention was employed consisted of 15 minutes of HIIT and then followed by 30 minutes of other sports activities leading to a non-significant impact on lipid profile (13). Moreover, a systemic review illustrated that HIIT had no significant influence on TC, LDL, and HDL levels, when compared with control group (26). Another review revealed that weekly energy expenditure of 1200-2200 Kcal through an aerobic exercise had a positive effect on lipid profile. Also, longer interventions and more aerobic activity sessions resulted in greater impact on lipid profile (2).

The MD+HIIT group demonstrated the most favorable changes in lipid profile. Total cholesterol, TG, and LDL-C levels decreased in this group, together with a non-significant increase in other variables, but TC changes in the MD+HIIT group were significant. Our findings are in line with other

researchers (3), reporting that exercise in women can decrease TG and LDL levels by 5% and 3%, respectively. Diet and exercise together were shown to be more effective to lower LDL level when compared to other interventions alone. To improve cardiometabolic health factors in obese individuals, a 9-month MD together with HIIT were demonstrated to be effective (27). Consistent with these results, another study revealed that MD together with moderate- to high-intensity aerobic exercises were more efficient than MD alone in reducing the indices of metabolic syndrome (17). Therefore, this combination seems to have a unique capacity to positively influence lipid profile.

In the present study, lipid profile including TC, TG, LDL-C, and HDL-C levels exhibited a higher degree of resistance to the interventions, highlighting the challenges in modifying lipid profile through short-term interventions. All intervention groups of our study demonstrated a significant and comparable decrease in HbA1c level suggesting that HbA1c level was highly responsive to the interventions. It was shown that MD can improve cardiometabolic biomarkers of insulin resistance and HbA1C (28, 29). MD was demonstrated to lower fasting blood glucose, insulin, and TG levels too (6).

MD is considered a nutrient-dense diet with a low glycemic index that is found in plant-based components including high intake of fruits, vegetables, and whole grains, and with a low intake of sweets that can positively affect glucose metabolism and the associated biomarkers. In a cross-sectional study involving type 2 diabetic patients, those with lower HbA1c level (<7%) exhibited better adherence to MD when compared to those with higher HbA1c levels that highlights the potential benefits of MD in glycemic control (28). Another cross-sectional study revealed that a high MD score was correlated with improved glucose control and reduced cardiovascular risk factors, including blood pressure and plasma lipids (29).

In our study, the HIIT group demonstrated a significant decrease in HbA1c level. This reduction along with an increase in skeletal muscle mass were described before (30), revealing the contribution of body composition in decrease of HbA1c in HIIT group. Improved body composition was illustrated to be associated with a lower lipid profile and blood pressure, as well as control of blood glucose in patients with Type 2 Diabetes Mellitus (T2DM) (31). Exercise was shown to play a crucial role in delivery and transport of glucose across muscular tissue, improvement of intramyocellular metabolism, and enhancing of insulin sensitivity and post-exercise glucose disposal (32).

Tjonna *et al.* found that following a HIIT protocol can lead to a greater increase in mitochondrial and glucose transport protein-4 (GLUT-4) content in muscular cells in comparison to MICT (33). HIIT, by increasing the expression of genes for GLUT4 and peroxisome proliferator-activated receptor coactivator-1 (PGC-1 α) can improve mitochondrial biogenesis and glucose transport, thereby enhancing oral glucose tolerance level (34). In contrast, when resistance training of either HIIT or MCT was employed for T2DM patients for one year, changes in HbA1c level and body fat indices were non-significant (32).

Our controlled trial design that allowed a systematic evaluation of the effects of MD, HIIT, and their combination on various cardiovascular risk factors had some limitations. It is crucial to acknowledge that confounding factors, particularly unmeasured variables, could not be completely eliminated. Compensatory eating responses to the HIIT exercise protocol could be a potential confounding factor that can affect lipid profile. The use of self-reported questionnaires to assess eating habits had the possibility of under- or over-reporting data. This may impact the accuracy of dietary information and influence the outcomes. Our study did not comprehensively assess LDL and HDL atherogenicity and function including factors such as particle size, composition, resistance to oxidation, and cytotoxicity.

Conclusion

HbA1c level was significantly responsive to MD and HIIT, while lipid profile changes were non-significant. Despite the non-significant changes, MD together with HIIT demonstrated the most favorable effect on LDL-C and TG levels.

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Authors' Contribution

MR and MKJ contributed to the study design and concept. MR, MS, and RT contributed to data collection. MR and FD contributed to data analyses.

RT and MR contributed to the interpretation of data. RT, MR, FD, MKJ and MS contributed to drafting and reviewing the final manuscript. All authors read and approved the final manuscript for publication.

Conflict of Interest

None declared.

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