Effects of Different Intensities of Circuit Resistance Training on Plasma level of High-Density Lipoprotein Subfractions and Apolipoprotein M in Untrained Young Men

ABSTRACT

Background and Objectives: Coronary artery disease (CAD) is the leading cause of death worldwide. It is well established that low level of high-density lipoprotein-cholesterol (HDL-C) is a strong and independent risk factor for CAD. Apolipoprotein M (apoM) is a component of HDL, which is involved in pre-β-HDL formation and cholesterol efflux to HDL. It is believed that resistance and aerobic exercise can significantly reduce risk of cardiovascular disease, especially by increasing serum levels of HDL-C. However, little is known about effects of these activities on HDL-apoM levels. The aim of this study was to investigate effects of circuit resistance training at different intensities on HDL-associated apoM levels in young untrained men.

Methods: Forty-five age- and weight-matched healthy untrained men were randomly assigned to a control group (n=10) and four training groups: 20% 1-repetition maximum (1RM) (n=9), 40% 1RM (n=8), 60% 1RM (n=7) and 80% 1RM (n=8). The subjects performed circuit resistance training consisting of barbell bench press, underarm flab, seated barbell curl, triceps exercise with chains, lying leg curl, squats, hyperextension, abs workout, sit-ups and quadriceps workouts (30 seconds each) in three bouts without rest between stations and with active rest (3 minutes) between sets or bouts. The training protocol was carried out for 43 minutes per session, three sessions a week, for five weeks.

Results: After the last training intervention, the exercise groups had higher apoM levels in total HDL and HDL-2 compared to the control group (P<0.05). However, no significant difference in HDL-associated apoM level was observed between the study groups.

Conclusion: The results of this study indicate that various intensities of circuit resistance training can alter HDL-associated apoM levels. The decreased HDL-3-associated apoM level could indicate increased rate of apoM transfer to HDL-2, which could potentially prevent development of atherosclerosis and CAD by enhancing the antioxidant effects of HDL.

Keywords: Circuit Resistance Training, Total HDL-M, HDL3-M, HDL2-M.
INTRODUCTION

Homeostasis imbalance is one of the main causes of heart attack (1). Coronary artery disease is the leading cause of mortality in the world (2). Coronary artery disease and metabolic syndrome are common among Iranians (3). Studies have indicated a reverse and significant relationship between high-density lipoprotein-cholesterol (HDL-C) levels and risk of atherosclerosis (4). Atherosclerosis is a chronic inflammatory condition that occurs when cholesterol ester accumulates in macrophage foam cells due to the inability of the cells to remove excess cholesterol (5). In ultracentrifugation, human HDL can be separated into HDL2 and HDL3 based on their density. HDL2 and HDL3 can then be separated into HDL2b and HDL2a, HDL3a, HDL3b and HDL3c (6). HDL has antiatherogenic properties and plays a critical role in reverse cholesterol transport. It can induce endothelial synthesis of nitric oxide and prevent low-density lipoprotein (LDL) oxidation and inflammatory responses. Proteolytic analyses have revealed presence of 56 HDL-associated proteins, including apolipoproteins and lipid transfer proteins, but the exact function of these proteins is still not fully understood (7). Apolipoprotein M (apoM) is a novel HDL-associated human apolipoprotein, which is also found in triglyceride-rich lipoproteins and LDL (8). Although apoM is present in only 5% of HDL particles, recent studies have demonstrated a positive correlation between plasma apoM levels and HDL-C concentrations. This indicates that apoM might be involved in HDL metabolism. ApoM plasma is mainly associated with α-HDL and affects its interconversion to pre-β-HDL particles. It has been demonstrated that a 2-fold increase in apoM concentration can decreases atherosclerosis progression (9).

The positive effects of exercise on factors associated with cardiovascular disease are well established. However, most of these studies have been focused on the effects of endurance training, and little attention has been given to the effect of resistance training (10). Currently, it is not clear how HDL and its components will respond to different intensities of resistance training. Therefore, the purpose of this study was to determine effects of different intensities of circuit resistance training on plasma level of HDL, HDL subfractions and apoM in untrained young men.

MATERIALS AND METHODS

We carried out this semi-experimental study on 45 non-active male students who were studying at the Golestan University of Medical Sciences in Gorgan (Iran). Mean age and weight of subjects was 19.52 ± 0.96 years and 60 ± 0.96 Kg, respectively. Study procedures were explained in detail, and then written informed consent was taken from all participants. Exclusion criteria included having drug/alcohol addiction, regular exercise activity in the past six months, a history of diabetes, kidney, liver and cardiovascular diseases, and any type of physical disability. The subjects were randomly divided into five groups: control (n=10), 20% one-repetition maximum (1RM) (n=9), 40% 1RM (n=8), 60% 1RM (n=7) and 80% 1RM (n=8) (Table 1).

The training protocol was designed based on a previous study (11). The subjects first became familiar with the training environment and equipment. Value of 1RM of the intended movements (bench press, seated cable row, arm cable curl, triceps cable curl, lying leg curl, barbell squat, lumbar extension, abdominal, decline sit-up and quadriceps) was calculated using the following formula and through trial and error:

\[ 1RM = \frac{\text{displaced weight}}{n - (\text{repit}2) \times 25\%} \]

After warm up, the subjects performed the movements in 10 stations at different intensities (20, 40, 60 and 80% of 1RM) for 30 seconds without rest between the stations. The exercises were performed in three sets with three active rest periods between each set. Blood samples (10 ml) were taken from subjects' forearms in a comfortable sitting position after at least eight hours of overnight fasting. Sampling was done 48 hours before the first training session and 48 hours after the last training session. The samples were collected in EDTA-coated tubes. Plasma was separated by centrifugation at 1500g for 15 minutes and then stored at -70 °C. were measured using the Prestige 24i automated analyzer (Japan). The mg/ml, respectively. The rest of the parameters normality assumption. Data were analyzed the measurement method.
ApoM was measured using commercial ELISA kits. HDL-C was measured by photometric method (ParsAzmun Co., Iran). The coefficient of variation and sensitivity of were 0.81% and 1%.

Kolmogorov–Smirnov test was used to test the normality assumption for all variables. ANOVA with repeated measures, the effect of time, group and their combination had no significant impact on the variables except for HDL3 (Table 3). Regarding the results of the ANOVA with repeated measures, the effect of time was significant in comparison with the control group before and after (P=0.021).

**RESULTS**

ApoM level in HDL2 and total plasma HDL increased significantly in all exercise groups and decreased in the control group. Table 3 shows alteration of apoM, HDL2 and HDL3 in the study groups.

Results of repeated measures ANOVA showed that, group and their combination had no significant impact on the variables except for HDL3 (Table 3). Regarding the results of the ANOVA with repeated measures, the effect of time was significant in comparison with the control group before and after (P=0.021).

### Table 1 - Characteristics of subjects in each group

| Variable                  | Group | Time | Control | 20% one-repetition maximum (1RM) | 40% 1RM | 60% 1RM | 80% 1RM |
|---------------------------|-------|------|---------|----------------------------------|---------|---------|---------|
|                           |       |      | Mean ± standard | error | Mean ± standard | error | Mean ± standard | error | Mean ± standard | error |
| Age (years)               |       |      | 20 ±1 | 19 ±1 | 19 ±1 | 19 ±1 | 18 ±1 | 18 ±1 |
| Height (cm)               |       |      | 177 ±8 | 178 ±3 | 176 ±6 | 183 ±7 | 181±4 |
| Weight (Kg)               |       | Pretest | 76 ±17 | 77 ±20 | 76 ±16 | 81 ±13 | 81 ±15 |
|                           |       | Posttest | 77±16 | 75±19 | 76±16 | 80±12 | 81±15 |
| Body mass index (Kg/m²)   |       | Pretest | 24±4 | 24±6 | 24±3 | 24±2 | 24±5 |
|                           |       | Posttest | 25±4 | 23±5 | 24±3 | 23±2 | 24±4 |
| Back Extensions (Kg)      |       | Pretest | 24±5 | 21±5 | 18±6 | 18±10 | 18±8 |
|                           |       | Posttest | 41±7 | 49±11 | 50±11 | 41±15 | 49±8 |
| Abdomen (Kg)              |       | Pretest | 28±6 | 22±8 | 25±6 | 24±7 | 21±10 |
|                           |       | Posttest | 41±9 | 50±23 | 56±17 | 62±13 | 49±15 |
| Back arm (Kg)             |       | Pretest | 42±7 | 31±5 | 37±7 | 44±15 | 35±6 |
|                           |       | Posttest | 47±8 | 47±9 | 50±11 | 53±7 | 48±4 |
| Barbell Bench Press (Kg)  |       | Pretest | 30±6 | 26±11 | 28±10 | 33±11 | 24±10 |
|                           |       | Posttest | 33±9 | 36±9 | 42±6 | 40±10 | 37±8 |
| Leg squats (Kg)           |       | Pretest | 57±11 | 54±15 | 53±12 | 64±14 | 54±11 |
|                           |       | Posttest | 69±22 | 75±20 | 90±15 | 83±21 | 91±16 |
| Leg (Kg)                  |       | Pretest | 95±35 | 145±209 | 61±14 | 63±12 | 61±11 |
|                           |       | Posttest | 121±59 | 177±59 | 163±36 | 148±27 | 181±39 |
| Front arm (Kg)            |       | Pretest | 16±3 | 13±5 | 11±5 | 14±6 | 11±6 |
|                           |       | Posttest | 21±6 | 24±3 | 27±4 | 30±7 | 28±4 |
| Back foot (Kg)            |       | Pretest | 21±2 | 23±9 | 16±5 | 19±2 | 18±5 |
|                           |       | Posttest | 21±5 | 23±5 | 24±3 | 28±4 | 24±3 |
| Front leg (Kg)            |       | Pretest | 34±7 | 36±12 | 31±12 | 38±4 | 35±10 |
|                           |       | Posttest | 39±8 | 48±13 | 48±6 | 53±7 | 52±7 |
| Armpit (Kg)               |       | Pretest | 49±6 | 47±8 | 44±5 | 48±6 | 44±6 |
|                           |       | Posttest | 46±7 | 46±7 | 51±6 | 52±7 | 50±4 |
| Total power (Kg)          |       | Pretest | 324±59 | 349±80 | 328±57 | 364±73 | 325±66 |
|                           |       | Posttest | 483±81 | 578±124 | 582±98 | 596±77 | 613±90 |

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DISCUSSION

The results of this study showed that five weeks of circuit resistance training at different intensities could alter some of the risk factors of cardiovascular disease. Although extensive research has been done on the impact of endurance and strength training on lipoprotein and lipid levels (12), there is little known about the effect of circuit resistance training on the level of HDL-associated apoM. In our study, HDL-apoM and HDL2-apoM increased in all training groups and decreased in the control group. In addition, HDL3-apoM level decreased after the 5-week training program. There is an inverse relationship between HDL-C levels and risk of atherosclerosis. HDL-C have been attributed to its role in reverse cholesterol transport (RCT). On the other hand, HDL-C changes may be dependent to changes in the concentration of its subclasses, particularly HDL2. Among all HDL subtypes, HDL2 is more effective in cholesterol delivery to hepatocytes (13). In our study, the exercise program exhibited favorable effects by increasing apoM and HDL2 and decreasing HDL3. Apolipoproteins are members of the lipocalin family with a mean plasma level of 0.9% in the healthy population (14, 15). According to human tissue expression array studies, apoM is predominantly present in adult liver and kidney. Liver-derived apoM is mainly secreted into the plasma and accumulates in lipoproteins, while kidney-derived apoM is connected to a multiligand, endocytic receptors megalin in the renal proximal tubule (16). Nevertheless, mechanisms that regulate transcription of the human apoM gene are not well-understood (17). Recently, it has been shown that plasma level of apoM has a positive correlation with plasma leptin levels and a negative correlation with cholesterol levels in obese individuals. In fact, it has been suggested that leptin can stimulate hepatic apoM expression (18). According to Lappalainene et al., intensive isokinetic exercise can significantly reduce plasma leptin levels and a negative correlation with cholesterol levels in obese individuals. In fact, it has been suggested that leptin can stimulate hepatic apoM expression (18). According to Lappalainene et al., intensive isokinetic exercise can significantly reduce leptin levels (19). Hence, it can be assumed that a possible decrease in leptin levels through resistance training could contribute to the apoM reduction. ApoM can delay LDL oxidation, alter HDL metabolism by increasing the production of pre-β-HDL and exert anti-atherosclerotic effects (9, 21). There is a strong correlation between plasma apoM and cholesterol concentrations, but the mechanisms of this association remain unknown (14). ApoM may facilitate the reverse cholesterol transfer process by converting small pre-β-HDL into larger pre-β-HDL (22). A study reported overexpression of the apoM gene in LDL

### Table 2- Plasma levels of apoM and lipoproteins before and after the training intervention

| Variable                  | Group          | Time   | Control     | 20% 1RM     | 40% 1RM     | 60% 1RM     | 80% 1RM     |
|---------------------------|----------------|--------|-------------|-------------|-------------|-------------|-------------|
|                           |                | Mean ± standard | Mean ± standard | Mean ± standard | Mean ± standard | Mean ± standard | Mean ± standard |
|                           |                | error     | error       | error       | error       | error       | error       |
| ApoM in plasma            | Pretest        | 7.85 ± 0.95 | 9.74 ± 5.13 | 8.83 ± 3.64 | 7.79 ± 5.97 | 6.99 ± 2.63 |
| HDL (mg/dl)               | Posttest       | 7.72 ± 0.84 | 10.8 ± 5.42 | 9.69 ± 5.24 | 8.49 ± 1.49 | 8.31 ± 1.43 |
| HDL3 (mg/dl)              | Pretest        | 0.82 ± 0.07 | 0.74 ± 0.11 | 0.60 ± 0.07 | 0.74 ± 0.13 | 0.76 ± 0.12 |
|                           | Posttest       | 0.73 ± 0.06 | 0.70 ± 0.08 | 0.76 ± 0.10 | 0.70 ± 0.16 | 0.74 ± 0.07 |
| HDL2 (mg/dl)              | Pretest        | 6.78 ± 0.91 | 8.99 ± 5.01 | 8.30 ± 3.96 | 7.93 ± 8.16 | 6.23 ± 2.72 |
|                           | Posttest       | 6.64 ± 0.54 | 10.04 ± 5.35 | 9.34 ± 5.70 | 8.60 ± 1.35 | 7.57 ± 1.48 |

### Table 3- Comparison of the effect of time, group and the interaction between time and group on biochemical variables

| Variable      | Effect of time | Effect of group | Effect of time and group |
|---------------|----------------|-----------------|--------------------------|
|               | F              | P-value         | F                        | P-value         | F              | P-value         |
| ApoM in plasma (μg/ml) | 2.559 | 0.12 | 0.807 | 0.53 | 0.311 | 0.868 |
| HDL3 (mg/dl)  | 5.925 | 0.021 | 0.439 | 0.779 | 0.807 | 0.53 |
| HDL2 (mg/dl)  | 1.788 | 0.193 | 0.228 | 0.92 | 0.803 | 0.534 |
receptor-knockout rats, which prevented progression of early atherosclerotic lesions (23). Furthermore, the protective activity of HDL against atherosclerosis could be related to its antioxidant and anti-inflammatory properties (21).

CONCLUSION

The 5-week circuit resistance training intervention at different intensities not only improved body mass index, muscle strength and weight loss, but also altered plasma concentrations of HDL, HDL subfractions and apoM. The reduction in HDL-3-associated apoM following the training program could be related to the increased exchange of apoM to HDL-2, which could potentially prevent development of atherosclerosis and coronary artery disease by enhancing the antioxidant activity of HDL.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article.

REFERENCES

1. Amouzad Mahdirejei H, Aghababaeian A, Mirsaeidii M, Fadai Reyhan Abadei S, Abbaspour Seyedi A. Effect of 8 weeks of resistance training on hemostasis indices and lipid profile in adult men. J Gorgan Unv Med Sci. 2014; 16 (2): 21-28. (Persian)
2. Alirezaei F, Ghanbarni-Niaki A, Josaghani H, Naghizadeh Ghomi M. Effect of Aerobic Interval Training on Plasma Apolipoprotein M Levels in Young Men. Medical Laboratory Journal. 2019; 13(3): 1-5.
3. Baratzaide Shokri M, Fathi R, Talebi R, Gorkani Safarzadeh A. Effect of 8 weeks aerobic training on plasma levels of apolipoprotein M in women with Normal weight and overweight women. Medical Journal of Mashhad University of Medical Sciences. 2014; 57(7): 852-858.
4. Ramezani H, Ghanbarni-Niaki A, Ahmazadeh A, Abdi A. Abad Rooh, Ashitani CH. The effect of twelve sessions of circular resistance training along with its protein consumption on surfaces in non-athletic men, HDL-M, total rest and lipolytic profile of apolipoprotein. Medical Journal of Mashhad University of Medical Sciences. 2014; 57(7): 852-858.
5. Rashidlamar A. Investigation of the effect of Aerobic and resistance exercises on peripheral blood mononuclear cells abcg1 gene expression in female athletes. J Shahid Sadoughi Univ Med Sci. 2012; 20(1): 1-9.
6. Lund-Katz S, Liu L, Thualnai S, Phillips M. High Density Lipoprotein Structure. Frontiers in Bioscience. 2003; 8: 1044-1054.
7. Lu B, Wu Ch, Zhao S. Plasma apolipoprotein O level increased in the patients with acute coronary syndrome. Journal of Lipid Research. 2012; 53: 1952-1957.
8. Luo G, Zhang X, Nilsson-Ehle P, Xu N. Apolipoprotein M, Lipids Health Dis. (2004) ; 3: 21.
9. Christoffersen C, Jauhiainen M, Moser M, Porse B, Ehnholm C, Boesl M. Effect of apolipoprotein M on high density lipoprotein metabolism and atherosclerosis in low density lipoprotein receptor knock-out mice. J Biol Chem. 2008; 283(4): 1839-1847.
10. Sheikholeslami Vatani D, Ahmadi S, Mojtabehed H, Marandi Mohammad Ahmadi Dekhsheid K, Faraji H, et al. Effect of mild and severe resistance exercises on cardiovascular risk factors in non-athletic students. Kosar Medical Journal. 2011; 16(2): 121-115.
11. Ghanbarni-Niaki A. Aghababaeian A. Effect of Various Intensities of Circuit Resistance Training on Plasma Levels of High-Density Lipoprotein-Associated Apolipoprotein O Total Cholesterol and Triglyceride in Untrained Men. Medical Laboratory Journal, Jul-Aug (in press), 2019; 13(4):
12. Ghanbarni Niaki A, Ali Akbari Bidokhti M, Saeedi A, Ardershiri S, Naghizadeh Qomi M. Effect of short-term resistance training with and without plant propagation of saffron on fat and lipid concentration Plasma Lipoprotein in Young College Students. Applied Sports Physiology Research Report.2014; 12: 24.
13. Ghanbarni-Niaki A, Fathi R, Ramroudi S, Hedayati M. Effect of 8 Weeks Endurance Training With Two Different Durations on Plasma HDL-Ghrelin in Male Rats. Iranian Journal of Endocrinology and Metabolism. 2011; 13(3): 309-314.
14. Ahnstrom J. Apolipoprotein M-Studies of Structure and Function. Lund University. 2009; 76.
15. Sevvana M, Ahnström J, Egerer-Sieber C, Lange HA, Dahlbäck B, Muller YA. Serendipitous Fatty Acid Binding Reveals the Structural Determinants for Ligand Recognition in Apolipoprotein M.
16. Nielsen L, Christoffersen Ch, Ahnstrom J, Dahlback B. ApoM gene regulation and effects on HDL metabolism. Trends Endocrinol Metab. 2009; 20(2): 66-71. doi: 10.1016/j.tem.2008.11.003.
17. Mosialou L, I. Zannis V, Kardassis D. Regulation of Human ApolipoproteinM Gene Expression by Orphan and Ligand-dependent Nuclear Receptors. The Journal of Biological Chemistry. 2010; 285(40): 30719-30730.
18. Xu N, Nilsson-Ehle P, Ahren B. Correlation of apolipoprotein M with leptin and cholesterol in normal and obese subjects. Journal of Nutritional Biochemistry. (2004); 15(10): 579-582.
19. Lappalainen Z, Nilsson-Ehle P, Lappalainen J, Ahnström J. Time-of-day effects during acute isokinetic exhaustive eccentric: Serum leptin response. Isoknetics and exercise Science. 2009; 17: 19-25.
20. Wei J, Shi Y, Zhan X, Feng Y, Luo G, Zhang J, et al. Estrogen upregulates hepatic apolipoprotein M expression via the estrogen receptor. Molecular and Cell Biology of Lipids. 2011; 1811(12):1146-1151.
21. Huang X-S, Zhao S-P, Hu M, Luo Y-P. Apolipoprotein M likely extends its anti-atherogenesis via anti-inflammation. Medical Hypotheses. 2007; 69(1): 136-140.
22. Mulya A, Seo J, Brown A, Gebre A, Boudyguina E, Shelness G. et al. Apolipoprotein M expression increases the size of nascent pre HDL formed by ATP binding cassette transporter A1. Journal of Lipid Research. 2010; 51(3): 514-524.

23. Dahlback B, Nielsen LB. Apolipoprotein M affecting lipid metabolism or just catching a ride with lipoproteins in the circulation? Cell Mol Life Sci. 2009; 66(4): 559-64. doi: 10.1007/s00018-009-8764-8.