

# The Effects of Short-term Circuit Resistance Training with Supplementation of Milk, Cheese Juice, Whey Protein on Resting Level Apolipoprotein M- HDL Total Cholesterol, Lipid Profile, Gastric Inhibitory Polypeptide and Plasma Estradiol in Untrained Young Men

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## ABSTRACT

Cardiovascular diseases are the most significant factors leading to death. This quasi-experimental study was carried out on 28 non athletic men. Subjects were divided into 4 groups. To the first group water (400 cc) was given, the second group received whey protein supplementation (50g), the third group had cheese juice (50g), and milk was given to the fourth one (400 cc), immediately after the training session. High-density lipoprotein (HDL), its sub fractions HDL2 and HDL3 cholesterol, apolipoprotein M, gastric inhibitory polypeptide and estradiol were evaluated prior to and after training for 12 sessions, three sessions in each week. The differences were not significant for gastric inhibitory polypeptide (GIP) and estradiol in the fourth group after training in comparison with the first phase. A significant decrease in apolipoprotein M- HDL total cholesterol on resting level were observed in all four groups and in HDL3 cholesterol with cheese juice and also whey protein in comparison with prior phase (P-values were 0.02, 0.001, 0.000, 0.015, respectively) (0.01, 0.01). A significant increase in HDL2 cholesterol level was observed in the groups with cheese juice and whey protein in comparison with their prior phases (P-values were 0.02, 0.03, respectively). This study showed that circuit resistance training has positive effects on the lipid profile changes in untrained young men.

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## INTRODUCTION

Obesity is considered as a risk factor for, cardiovascular diseases specially because it increases blood cholesterol and results in high blood pressure. Overweight and obesity are multi factorial phenomena in which genetic and environmental factors

as well as lifestyle are involved. In fact, in developed countries physical inactivity and poor diet are the causes of obesity and cardiovascular diseases (Dridi *et al.*, 2009). Resistance training, in addition to increasing strength and muscle mass, can have beneficial effects on risk factors associated with cardiovascular diseases and can contribute to the effective prevention of cardiovascular diseases (Sheykholeslami *et al.*, 2009). Human studies have indicated a significant inverse relationship between HDL-C levels, the major protein apolipoprotein A-I (APOA-I) and heart-coronary diseases (atherosclerosis) (Ghanbari-Niaki *et al.*, 2007; Khabazian *et al.*, 2009; Khabazian *et al.*, 2008).

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The role of HDL-C in the prevention of cardiovascular disease through the transfer of excess cholesterol from peripheral cells and carrying it to the liver is exerted during a process called reverse cholesterol transport (RCT) (Khabazian *et al.*, 2008). The apolipoprotein constituents are made from the major plasma lipoproteins which regulate lipoprotein metabolism and determines the unique role of these lipoproteins in cholesterol transport and lipid metabolism (Davidsson *et al.*, 2010; Dominiczak *et al.*, 2011).

Apo lipoprotein M (Apo-M) is a novel human protein of apolipoprotein classes with 188 amino acids that are identified, characterized and highly expressed in liver and kidney tissues. Apo-M is mainly associated with HDL-C in human plasma small amounts of which are also available in rich triglyceride lipoprotein and low-density lipoproteins (LDL) (Todiodi *et al.*, 2007).

HDL particles containing Apo-M isolated from human plasma are of two types in size, weight and density. Lipid composition of human HDL containing Apo-M is similar to total HDL (Sevvana *et al.*, 2007). The level of Apo-M in plasma/serum is affected by several factors such as pregnancy, hyperglycemia, plasma leptin concentration, obesity, diabetes, and insulin concentration (Kujiraoka *et al.*, 2013; Lamant *et al.*, 2006; O'Bryan *et al.*, 2004; Xu *et al.*, 1999; Segrest *et al.*, 2013; Song *et al.*, 2012; Ghanbari Niaki *et al.*, 2007). In human plasma, 5% of the HDL particles contain Apo-M. Although there is not Apo-M in all HDL particles, investigation has shown that they are available as positive association between plasma levels of Apo-M and HDL-cholesterol concentrations in humans (Christoffersen *et al.*, 2008). Estrogens are a group of steroidal compounds containing estrone, estradiol, and estriol that act as the growth regulating hormones and reproduction hormones (Todiodi *et al.*, 2008).

Studies showed that the prevalence of cardiovascular disease in postmenopausal women who received estrogen alone or in combination with progesterone was less than other treatments. In this respect it seems that hormone 17 beta-estradiol (estrogen) may reduce low-density lipoprotein (LDL), and may lead to increased levels of HDL-C, increased coronary vasodilatation, improved glucose metabolism and decreased plasma insulin levels, which are important for the prevention of heart disease (Babiker *et al.*, 2009). Gastric inhibitory polypeptide (GIP) and gastric inhibitory polypeptide-1 (GIP-1) are known as important intestinal hormones that are essential for normal glucose homeostasis (Ugleholdt *et al.*, 2009). Few studies have been carried out on the GIP effects on cardiovascular system. However, studies on the effects of GIP in the regulation of blood flow have been reported. Also, referring to the endothelium, the results have shown that GIP production of nitric oxide from endothelial cells stimulates the portal vein (Nie *et al.*, 2012).

The effects of different types of regular aerobic exercises and, to some degree, those of resistance training on plasma lipids, lipoproteins, particularly on high density-lipoprotein (HDL) and its compositions have been well documented. However, the effects of circuit resistance training (CRT) with or without milk, Cheese juice, whey protein products on the resting level apolipoprotein M-HDL total cholesterol in, lipoprotein levels, and GIP and plasma

estradiol have not been studied yet. This study was conducted to investigate the effect of a 4 week- CRT program with or without supplementation of milk, cheese juice, whey protein on resting level apolipoprotein M- HDL total cholesterol, lipoprotein levels, and GIP and plasma estradiol in untrained young men.

## MATERIALS AND METHODS

The study was approved by the ethics committee of the University of Mazandaran (UMZ) and Babol University of Medical Sciences (BUMS) in Mazandaran, Iran in accordance to the declaration of the Iranian Ministry of health.

### Subjects

A written consent was obtained from all 28 young male and untrained high schools students (age  $21.6 \pm 0.44$  y, height  $172.66 \pm 1.45$  cm, body weight  $70.8 \pm 6.5$  kg, and body mass index (BMI) of  $20.19 \pm 1.70$  kg/m<sup>2</sup>) without any weight circuit-resistance training experiences who were employed and randomly assigned to four groups: training-water (TW, n=7), training-milk (TM, n=7), training- cheese juice (TCJ, n=7), and training- whey protein (TWP, n=7). Subjects received either tap water (400 cc) or low fat milk (400cc), or whey protein supplementation (50g) or cheese juice (50g), immediately after training sessions. It should be noted that the solution has gradually been administrated.

### Training protocol and Statistical analysis

The circuit resistance training was performed according to Ghanbari Niaki (2006) and Ghanbari Niaki *et al.*, (2007) with a little modification in the number of exercises (bench press-off pins, inclining bench press, cable push-down, dead lift halter, squat 90° over neck, Roman chair back extension, crunch with free weight, leg press, leg extension, seated calf raise). Subjects were asked to perform circuit resistance training for 45-50 minutes/session, 3 days/week and for 4 weeks. Each training session consisted of warm-up (10min), cool-down (10min), and circuit resistance of 10 exercises (25 seconds for each exercise, 8-12 repeats, and 2 rounds/session with 5 minutes rest period between rounds) to strengthen most of the large muscle groups. Each load corresponded to 60% of one repetition maximum. All exercises were conducted with the use of free weight and machine. The subjects were instructed to follow a normal lifestyle, to maintain daily habits, to avoid any medications, and to refrain from exercise 3 days before the blood sampling.

Blood samples were obtained from an antecubital vein 72 hours before and 72 hours after the last circuit resistance training session while the subjects fasted overnight (at least 12h). Plasma samples were immediately frozen and stored at  $-20^{\circ}\text{C}$  and  $-80^{\circ}\text{C}$  for subsequent analyses (within 4-6 weeks). Plasma Apo-M was determined by using a commercial human Apo-M, ELISA Kit (Catalog Number-CSBEL001947HU, CUSABIO-BIOTECH, China) with detection range of 1.56 ng/ml-100ng/ml, and the sensitivity of 0.39 ng/ml. To measure HDL3 cholesterol sedimentary method and enzymatic assays were used with Pars

azmon company kits/ Iran. HDL2 levels obtained HDL2= -HDL3 method. HDL2 cholesterol levels were determined using the difference between HDL and HDL3 cholesterol. GIP Plasma level was measured by Elisa immunoassay enzyme method for which Cusabio Biotech company kits/China were used. Plasma estradiol concentration was determined by ELISA (Diagnostics Biochem Canada Inc., Ontario, Canada). After normalization of the data by suitable test, dependent and independent t-tests were employed. All the data were reported as mean  $\pm$  SE. A significant difference was accepted at alpha  $P \leq 0.05$ . All statistical analyses were performed with SPSS (Version 18; SPSS, Chicago, IL).

### Preparation of cheese juice extract

At first 3 kg of milk was heated at the temperature of 100 degrees, then the heat was reduced to prevent it from reaching its boiling point. After that from a mixture of 50 grams of vinegar with 10 grams of salt and 50 grams of yogurt, which were already prepared, were gently added to the surface of the boiling milk with a spoon. In this case milk is divided into two layers, the upper layer was cast away and the solid part after filtration and dewatering was kept in the fridge. After each training session, 50 grams of it would be mixed with water and consumed.

## RESULTS AND DISCUSSION

The densities of the measured variables are shown in Table 1. Data analysis showed that apolipoprotein M- HDL total cholesterol levels in 4 groups significantly decreased, while HDL2-C and HDL3-C levels in TCJ and TWP groups had significant increase and decrease, respectively (P-values were 0.02, 0.03, 0.01, 0.01, 0.001, 0.002, 0.001, and 0.004, respectively) (Table 1).

The above data provided some support for the hypothesis that physical activity and exercise could be utilized to improve Cholesterol levels. Regular physical activity has been shown to increase HDL2-C cholesterol and to decrease HDL3-C and apolipoprotein M- HDL total cholesterol levels significantly.

Despite various studies on the effect of exercise on the levels of plasma apolipoproteins including apolipoprotein AI (Khabazian *et al.*, 2009), no study has been carried out to examine the effect of exercise on the levels of Apo-M. The results of the present study, however, showed that doing a 4-weeks resistance training reduced the levels of plasma Apo-M. Descriptive studies on human tissue have shown that Apo-M mainly in the liver and kidney and in smaller amounts can be found in the fetal kidneys and liver.

Thus, Apo-M may have a special function in the body, which may be associated with hepatic lipid metabolism and lipoprotein (Luo *et al.*, 2009). Recently it has been shown that in obese individuals, plasma levels of Apo-M is positively associated with plasma leptin level and negatively with the level of cholesterol. Indeed, it is suggested that leptin can stimulate liver cells to produce Apo-M. Moreover, leptin and its receptor are essential for the expression of Apo-M (Xu *et al.*, 2004).

**Table 1:** The mean  $\pm$  SE of serum concentration in pre-test and post-test.

Variable	Groups	Before Exercise	After Exercise
		Mean $\pm$ SD	Mean $\pm$ SD
Apolipoprotein M- HDL-c (ng /ml )	TW	10.26 $\pm$ 4.66	5.73 $\pm$ 2.48 *
	TM	20.53 $\pm$ 4.462	4.46 $\pm$ 1.11 *
	TCJ	17.43 $\pm$ 2.65	4.88 $\pm$ 1.42 *
	TWP	11.18 $\pm$ 5.26	2.80 $\pm$ 1.29 *
HDL2-C(mg/dl)	TW	16.07 $\pm$ 3.64	16.07 $\pm$ 3.88
	TM	19.38 $\pm$ 3.81	20.00 $\pm$ 2.97
	TCJ	14.47 $\pm$ 3.09	19.92 $\pm$ 2.53 *
	TWP	13.48 $\pm$ 4.31	18.66 $\pm$ 3.07 *
HDL3-C(mg/dl)	TW	23.51 $\pm$ 3.60	33.35 $\pm$ 5.34
	TM	30.30 $\pm$ 5.79	33.04 $\pm$ 4.31
	TCJ	30.14 $\pm$ 5.71	27.82 $\pm$ 4.74 *
	TWP	27.67 $\pm$ 5.55	25.98 $\pm$ 6.44 *
Estradiol (pg /ml)	TW	69.85 $\pm$ 14.67	66.00 $\pm$ 23.30
	TM	68.71 $\pm$ 17.63	60.42 $\pm$ 16.46
	TCJ	64.57 $\pm$ 20.80	65.28 $\pm$ 14.70
	TWP	55.28 $\pm$ 9.46	56.42 $\pm$ 14.02
GIP (ng /ml)	TW	42.37 $\pm$ 45.35	35.21 $\pm$ 22.28
	TM	32.90 $\pm$ 29.37	17.50 $\pm$ 8.63
	TCJ	27.45 $\pm$ 24.06	19.94 $\pm$ 12.58
	TWP	58.47 $\pm$ 53.40	31.90 $\pm$ 17.25

HDL: High-density lipoprotein cholesterol, HDL2 and HDL3 cholesterol, GIP: Gastric inhibitory polypeptide,  
Training cheese juice: TCJ, Training Milk: TM, Training-Water: TW, Training whey protein: TWP.  
(\* Inter a group difference at  $P \leq 0.05$ ).

In a study Lappalainen *et al.*, (2009) showed that eccentric intense isokinetic exercise significantly reduced leptin levels. Although in the present study the levels of leptin were not measured, the reduced leptin levels might have happened as a reduction mechanism of Apo-M. However, the study reported that estrogen in vivo and in vitro conditions by a series of mechanisms upregulated the Apo-M (Wei *et al.*, 2011). In this study, one of the possible reasons for low levels of Apo-M may be the decreased estrogen levels; So, the reduction of Apo-M in exercise and milk consumption group may be associated with estradiol further reduction.

In this study, levels of HDL2, increased significantly in the exercise groups with whey protein consumer and with cheese juice consumer in comparison to other groups, Although HDL3 level, increased significantly in the exercise groups with milk and water consumers in comparison with other groups. Indeed, a positive correlation between the levels of LDL cholesterol and cardiovascular disease was observed as well as an inverse relationship between HDL cholesterol and atherosclerosis (Ahnstrom *et al.*, 2009). HDL particles, through their anti-inflammatory and anti-oxidative activities act as anti-atherogenic agents. HDL particles, as a mediator for the return of tissue Cholesterol to the liver for bile secretion, protect against atherosclerosis.

Yktayar *et al.*, (2011), in their study on the effects of resistance training, endurance and combination on lipid profiles and non-athletic middle-aged men reported that resistance exercise increased the levels of HDL. Studies done by Ghanbari Niaki *et al.*, (2011), showed that a single bout of circle resistance exercise training increased the levels of plasma HDL-C in female students.

Teixeira *et al.*, (2012), in their study on the effect of resistance exercise with and without whey protein on biochemical parameters of lipid metabolism and risk of cardiovascular disease in animal models showed that resistance training combined with whey protein could improve the lipid profile, lower total cholesterol and non-HDL cholesterol but whey protein without resistance training significantly increased HDL cholesterol concentrations which is consistent with the present research. However, in this study, no significant changes in the levels of estradiol and estradiol were observed in the milk consumption exercise group and water consumption exercise group compared with the pre-test.

17 beta-estradiol hormone (estrogen) by reducing LDL, increasing HDL levels, increasing coronary vasodilatation, improving glucose metabolism and decreasing plasma insulin levels, may have an important role in preventing heart diseases. (Babiker *et al.*, 2009). It is reported that estradiol increases during exercise however, it is reduced significantly after exercise (Ives *et al.*, 2011). The results showed that although consumer resistance training and supplements (especially whey protein) caused a slight reduction in GIP plasma levels, the changes in GIP plasma in pre-test and post-test showed no significant difference between the study groups that is consistent with a number of studies (Mortensen *et al.*, 2012).

Solomon *et al.*, (2010) studied the effect of a diet with a low or high glycemic index along with aerobic exercise (walking on a treadmill or cycling for 60 minutes per session, 5 days a week with a maximum heart rate of 85% for 12 weeks) on 22 obese patients with pre-diabetes. The results suggested that GIP response after food intake with lower glycemic index and exercise was delayed while these changes were not observed in the group with a high glycemic index diet with. Also, one possible reason for low levels of GIP in this study, might have been a lack of adequate stimuli such as glucose. On the other hand, since the level of GIP increases while food intake, the decrease in the plasma levels of GIP may happen over time and by its decreased plasma level.

## CONCLUSION

The results showed that the combination of resistance training and protein composition in the milk group could affect lipid and lipoprotein profiles and could increase the levels of HDL particles which play an important role in the prevention of cardiovascular diseases.

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