The Effects of Combined Training on Interluken-6 and C Reactive Protein as Non-traditional Cardio Risk Factors in Inactive Students

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ABSTRACT

Studies have shown a positive association between regular physical activity and reduction of cardio risk factors. The objective of this research was to examine the effects of combined training on some cardio risk factors in inactive students. Thirty healthy inactive men were assigned into two groups: 1) combined training (CTG) (n=15), 2) no-training (NTG) (n=15). The CTG performed 5 resistance exercises and this was followed by 30 minutes of endurance training with 60% - 80% HRmax for 8 weeks (3 days per week). Meanwhile, the NT group continued the same activity routine that they had used prior to becoming a study participant. Pre- and post-measures included VO₂ max, muscle strength, body composition, and blood cardio risk factors. The VO₂ max and muscle strength significantly (P<0.05) increased in CTG. The percentage of body fat tended to slightly decrease (P>0.05) and skeletal muscle mass significantly increased in CTG (+0.85kg, P = 0.003). However, these parameters did not show any change in NTG. The concentrations of total cholesterol, TG and LDL in the blood did not significantly change during the study in the two groups (P> 0.05). HDL significantly improved after week 8 of combined training in the CTG. Furthermore, CRP did not change in the two groups (P> 0.05), while the level of interleukin-6 had decreased significantly in the CTG. The results of this study have shown that combined training improves some cardio risk factors, cardiorespiratory fitness, as well as muscle strength and body composition in inactive students.
INTRODUCTION
Cardiovascular diseases are among major diseases in the world, which are responsible for nearly 30% of global mortality rates (WHO, 2009). Physical inactivity is found to be the major independent risk factor for the development of cardiovascular diseases (Thompson et al., 2003). Several studies have shown that exercise has a significant impact on the so-called atherogenic dyslipidemia, which is characterized by high triglycerides, low high-density lipoprotein cholesterol (HDL-C) and small-dense low density lipoprotein cholesterol (LDL-C) particles (Tambalis et al., 2009; Cornelissen & Fagard, 2005).

Traditionally, many studies focused on endurance exercise for prevention or rehabilitation of cardiovascular diseases and there is a widespread acceptance of the benefits of regular endurance training in reducing the risks of cardiovascular diseases (Janssen & Jolliffe, 2006; Fahlman et al., 2002). In addition, a few studies have looked into the effects of resistance training on the risk factors and reported that by increasing muscle strength and muscle mass after resistance training, the percentage of body fat decreased, while there was an improvement in glucose tolerance, resting energy expended (REE) and insulin resistance (Schmitz et al., 2003).

Taking into consideration the positive effects of endurance and strength training on the prevention and improvement of certain cardiovascular diseases, there have been suggestions that these two types of training be combined (Pescatello et al., 2004; Sigal et al., 2004). In the recent decades, some studies have focused on the effects of combined training on cardiovascular risk factors, while cardiovascular disease patients (Lee et al., 2001) and diabetics (D’hooge et al., 2011) were the subjects of these studies. However, these studies did not carry out on inactive young people and also cover enough inflammatory biomarkers of the blood as non-traditional risk factors.

Apart from the traditional cardio risk factors, some researchers have also focused on the effects of exercise training on some inflammatory biomarkers, such as C reactive protein (CRP) and interleukin 6 (IL-6) that are known as non-traditional cardio risk factors (Balducci et al., 2010; Keller et al., 2005). These markers are acute phase proteins, in which their levels in blood increase in response to inflammation. These markers are recognized as potential tools in the prediction and detection of atherosclerosis and its complications because inflammation is occupied in mediating all stages of the atherosclerotic process (Pearson et al., 2003).

Some studies have indicated that physical activity level is inversely associated with plasma levels of IL-6 (Dekker et al., 2007) and CRP (Stewart et al., 2007), and these factors have been recommended for clinical assessments of cardiovascular risks (Brinkley et al., 2009). Meanwhile, some evidence has indicated that exercises have a
full anti-inflammatory effect (Dekker et al., 2007; Stewart et al., 2007). However, a few studies have been carried out on the effects of combined training on CRP and IL-6 and the results of these studies were found to be inconsistent (Martins et al., 2010; Oberbach et al., 2006).

According to WHO (2008), cardiovascular risk factors have increased among young people in the world, and hence, it is necessary to find new exercise protocols that are healthy and least costly for decreasing or the prevention of CVD. The purpose of this study was to investigate the effect of combined endurance and strength training on some cardiovascular risk factors, traditional and non-traditional, and fitness in inactive young men.

METHODS

Subjects

The sample of this study comprised 30 male students aged between 18 and 24 years, who had been invited to participate in the study. They were selected from among volunteer students (96 persons) at the beginning of the semester using a simple random sampling method. Those selected were then randomly assigned into one of the two groups, the combined training group (CTG) and no-training group (NTG).

The volunteers were young and healthy but inactive, i.e. and they had not participated in any regular physical activity during the preceding 6 months (< 2 days/week; <20 minutes/day). They were admitted for evaluation two days prior to the commencement of the exercise protocol. A physician at the Shomal University laboratory in Iran compiled the medical history and conducted the physical examination and simple screenings to gather demographic information and determine the eligibility of the volunteers. Specific exclusion criteria were: 1) History of cardiovascular disease, smoking, orthopaedic or diagnosed diabetes; 2) Currently engaged in physical activity at least 2 days/week for 20 minutes or more each time; 3) Currently participating in an organized weight management programme; and 4) Taking drugs that could modify exercise performance, metabolism or desire for food. The Shomal University in Amol, which is located in the north of Iran, was selected as the location for the study as the university sponsored the subjects and all the instrumentations for this research.

Assessment of the Body Composition and Performance Characteristics

Total body composition was determined using a Bioelectrical Impedance Analyzer (Inbody 320). Beck (2007) reported that the InBody 320 model could be used. Beck (2007) reported that the InBody 320 model is a valid instrument (R=0.99) for estimating the parameters related to body composition among adults with a normal to overweight BMI.

The subjects were fasting as a prerequisite four hours prior to the testing and also they did not perform any exercise 24 hours before the examination which was according to the guidelines of ACSM (Dwyer & Davis, 2008, pp. 56-57). Having their age, height and gender recorded, the
analyzer was adjusted and the subjects were asked to stand on the anterior and posterior electrode plates, with minimum clothing and without having their shoes on. The subjects were motionless on the device during test. At the end of the test, all the parameters were put out by the analyzer.

Blood pressure (BP) was measured using a digital sphygmomanometer and heart rate (HR) of rest and exercise was measured by electric HR monitor/watches (polar; Fs3c). These were measured for all the volunteers according to the standard guidelines outlined by the American College of Sports Medicine (Dwyer & Davis, 2008).

The subjects completed an all-out, progressive Bruce protocol on treadmill (indirect test) for estimating maximal oxygen consumption (VO$_2$max) (Bruce, Kusumi, & Hosmer, 1973). Given the maximum time in minutes that the subjects run on treadmill (T), VO$_2$max was calculated using the following formula for men:

$$\text{VO}_2\text{max} = 14.76 - (1.379 \times T) + (0.451 \times T^2) - (0.012 \times T^3).$$

One repetition maximal (1RM) was used for estimating dynamic muscle strength by weight machine (Model: Olympia, Italian). Both upper and lower body strengths were assessed using the bench press and leg press exercises, respectively. For research purposes, 1RM is recommended to estimate dynamic muscle strength (Maud & Foster, 2006, p. 129 & 141).

**Biochemical Analysis**

Blood samples were taken at baseline, prior to the beginning of the exercise programme, and at the end of 8 weeks of the training programme for the two groups. The volunteers fasted for 12 hours and fasting blood samples were taken by a trained nurse. They sat on chairs and standard venipuncture techniques were used to collect the blood from the antecubital vein. The total blood collection for each testing session was 20 cc per volunteer. Serum total cholesterol (TC), low density lipoprotein (LDL), high density lipoprotein (HDL), and triglyceride (TG) concentrations were measured using enzymatic methods.

Interleukin-6 was determined using an enzyme-linked immunosorbent assay (Human IL-6 Platinum ELISA, BMS213/2, eBioscience). C-reactive protein was determined by using quantitative sandwich enzyme immunoassay (Monobind Inc. Product code: 3175-300).

**Mixed Training Protocol**

For improvement of cardiovascular and muscle fitness, ACSM recommended a combination of aerobic activities and resistance exercise (Thompson, Gordon, & Pescatello, 2009, p. 154). The training programme included at least three days per week, with a duration of 20-30 minutes of aerobic activity (60-90% HRmax) and resistance training with 2-3 sets of 8-12 repetitions, as suggested by ACSM (Nieman, 2003, p. 258).
The combined training group performed exercise training protocol included the resistance training and endurance training for 8 weeks (3 alternating days during the week). The combined training group first performed the resistance training consisted of 5 exercises (the unilateral knee extension, unilateral knee flexion, double leg press, seated chest press and seated row) with weight machine for the lower and upper body with 3 sets of 8-12 repetitions for each resistance exercise with 2 minutes of rest between the sets. The target load of this particular protocol was 80% of 1-RM, which was achieved after 3 weeks of progressive resistance training. After estimating the 1-RM, each subject initiated the resistance training with 60% of 1RM for the lower body and trunk and 50% of 1-RM for the upper body which the load gradually increased to 80% of 1-RM. Once the four and eight weeks resistance training was completed, the 1RM tests were repeated (Toloee, 2012).

Endurance training was followed after the end of the resistance training in each session for 30-minutes. This training included 25-30 minutes of moderate to high intensity exercise (from 60% HRmax in week one to 85% in week 6) comprised of jogging and running on the track. Meanwhile, the exercise intensity of subjects during the sessions was monitored by Polar Beat heart rate monitor (polar; Fs3c). Five minutes of warm-up and five minutes of cool-up were performed by the subjects for all the major muscle groups before and after the training sessions (Toloee, 2012).

Procedures
After the selection of the final sample and prior to commencement of the study, the volunteers were informed of the study process. The subjects were required not to engage in any physical activity 2 days prior to attending the laboratory. For two days, they recorded their daily dietary intake through a questionnaire. All the measurements were gathered after a 12-hour, overnight fasting period. Within two days, i.e. prior to the commencement of the exercise programme, all the pre-test measurements of the subjects from the two groups were taken.

Following that, the combined training group participated in the special exercise programme. The exercise sessions were the same for each volunteer and held for a 3-day-a-week cycle (48–72 hours between sessions) for a total of 8 weeks. The subjects were refrained from engaging in any other physical exercise programme to minimize confounding variables. During this experimental period, the control group went about with their daily activities and remained inactive. After the 8-week training exercises, post tests were conducted on all the subjects. All the post-test measurement methods were similar to those of the pre-tests.

Statistical Analysis
SPSS.18 software was used for the data analysis. The descriptive statistics of the dependent variables were mean and standard deviation. An independent sample t-test analysis was used to compare the values
of the dependent variables in baseline and the post-test between the CTG and NTG. Paired Student’s t test, as a valid statistic method, was used to compare the baseline versus post intervention (post-test) values within the groups. A value of p<0.05 was considered to be statistically significant.

RESULTS

Body Composition Characteristics
Comparisons of body composition between baseline and post tests are presented in Table 1. The results of the analysis indicated that there were no significant differences between the CTG and the NTG in the baseline values of the body composition characteristics. However, there was a significant difference in the amount of baselines and after the combined training for skeletal muscle mass (Diff. Mean = 0.87kg, P = 0.002) and the percentage of body fat (Diff. Mean = -1.59%, P = 0.01). Similarly, there were no significant differences in the amount of baselines and after the combined training for weight and a waist-hip ratio. Furthermore, these parameters showed no significant differences in the amount of baseline and post-tests in the NTG (P >0.05).

Biochemistry Cardio Risk Factors and Blood Hemodynamic Variables
The results of the biochemistry cardio risk factors during the baseline and post-tests are shown in Table 2. All the blood variables were in the clinically specified normal ranges. There were no significant differences in baseline values of biochemistry cardio risk factors between the CTG and the NTG. There were significant differences in the amount of baselines and after the combined training for HDL (Diff. Mean = -2mg/dl, P = 0.002), and interleukin 6 (Diff. Mean = -0.23pg/dl, P = 0.003). The triglycerides tended to slightly decrease in the CTG (P=0.72). In addition, the triglycerides and HDL in the CTG were significantly lower.

TABLE 1
General and Body Composition Characteristics of the CTG and the NTG at Baseline and at the End of 8 Weeks of Training

<table>
<thead>
<tr>
<th></th>
<th>Mixed Training Group (n=14)</th>
<th>Control group (n=14)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>pre</td>
<td>Post test</td>
</tr>
<tr>
<td>Age (year)</td>
<td>19±1</td>
<td>-</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>175±4</td>
<td>-</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>71.3±13.6</td>
<td>71.44±13.86</td>
</tr>
<tr>
<td>Dietary Intake (kcal)</td>
<td>2392±102.5</td>
<td>2401.29±107.14</td>
</tr>
<tr>
<td>Skeletal Muscle Mass (kg)</td>
<td>32.42±5</td>
<td>33.30±4.87**</td>
</tr>
<tr>
<td>Percent Body Fat (%)</td>
<td>18.12±7.1</td>
<td>16.80±6**</td>
</tr>
<tr>
<td>Waist-Hip Ratio</td>
<td>.83±.05</td>
<td>.83±.05</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>23.42</td>
<td>23.35</td>
</tr>
</tbody>
</table>

Values are the mean ± standard deviation. * Significant change within group at p < 0.05. ** Significant change within group at p < 0.01.
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TABLE 2
The Comparison of Biochemistry Cardio Risk Factors between Baseline and at the End of 8 Weeks of Training in the CTG and the NTG

<table>
<thead>
<tr>
<th></th>
<th>Mixed Training Group (n=14)</th>
<th>Control group (n=14)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>pre</td>
<td>Post test</td>
</tr>
<tr>
<td>Total Cholesterol (mg/dl)</td>
<td>151±27.3</td>
<td>159.57±26.10</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>80.6±39.5</td>
<td>78.14±26.71†</td>
</tr>
<tr>
<td>Low Density Lipoprotein (mg/dl)</td>
<td>87.8±24.6</td>
<td>94.94±26.63</td>
</tr>
<tr>
<td>High Density Lipoprotein (mg/dl)</td>
<td>47±3.4</td>
<td>49.00±2.48**†</td>
</tr>
<tr>
<td>High sensitivity CRP (mg/dl)</td>
<td>1.02±1.02</td>
<td>.97±1.02</td>
</tr>
<tr>
<td>Interleukin-6 (pg/dl)</td>
<td>1.50±.42</td>
<td>1.26±.38**</td>
</tr>
<tr>
<td>Systolic Blood Pressure of Rest (mmHg)</td>
<td>128.1±15.1</td>
<td>126.21±14.41</td>
</tr>
<tr>
<td>Diastolic Blood Pressure of Rest (mmHg)</td>
<td>82±8.63</td>
<td>78.36±10.17</td>
</tr>
</tbody>
</table>

Values are the mean ± standard deviation. HOMA, Homeostatic Assessment Model. * Significant change within group at p < 0.05. ** Significant change within group at p < 0.01. † Significant difference between the CTG and NTG at p < 0.05. †† Significant difference between the CTG and NTG at p < 0.01.

TABLE 3
A comparison of some Fitness Parameters between Baseline and the End of 8 Weeks of Training in the CTG and the NTG

<table>
<thead>
<tr>
<th></th>
<th>Mixed Training Group (n=14)</th>
<th>Control group (n=14)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pre</td>
<td>Post test</td>
</tr>
<tr>
<td>Heart Rate at Rest (bpm)</td>
<td>79.3±11.9</td>
<td>77.00±13.78*</td>
</tr>
<tr>
<td>VO2max (ml. kg⁻¹. min⁻¹)</td>
<td>35.83±5.1</td>
<td>41.65±4.30**††</td>
</tr>
<tr>
<td>leg press (kg)</td>
<td>41.9±6.26</td>
<td>62.91±15.7**††</td>
</tr>
<tr>
<td>chest press (kg)</td>
<td>36.5±11.2</td>
<td>42.21±12.68**</td>
</tr>
</tbody>
</table>

Values are the mean ± standard deviation. *Significant change within group at p < 0.05. **Significant change within group at p < 0.01. † Significant difference between the CTG and NTG at p < 0.05. †† Significant difference between the CTG and NTG at p < 0.01.

(P = 0.023) than in the NTG during post-testing. However, a comparison between the baseline and post tests in both the groups revealed no significant differences in the total cholesterol, LDL, hs-CRP, systolic blood pressure and diastolic blood pressure (P>0.05).

Fitness Parameters

The results of the analysis showed that post training resting HR was significantly lower (Diff. Mean = -2.28, P = 0.023) than the baseline values for the CTG. Similarly, VO2max was also significantly higher (Diff. Mean =5.81, P = 0.001) than the baseline values in this group. There
were no significant changes in the NTG. Furthermore, there was a significant increase in leg press record (Diff. Mean = 20.97kg, p = 0.001) and chest press record (Diff. Mean = 17.94kg, P = 0.001) from the baselines to the post-tests in the CTG.

DISCUSSION

It was hypothesized that a mixed intervention of resistance and endurance training would improve some non-traditional cardio risk factors, together with lipid profiles, body composition and fitness parameters in inactive youth men.

In the current study, after 8 weeks of the combined training, IL-6 level showed a significant decline in the CTG. The findings of the present study are in line with those of Balducci et al. (2010) who indicated that IL-6 level significantly declined in the mixed resistance and aerobic training group after 10 months of exercise programmes in type 2 diabetic patients. In the study mentioned above, the duration of the exercise protocol was long (i.e. 10 months), but the training programme was only twice a week and the exercise intensity was also lower than the present study. The study also evaluated the effects of combined training for 12 weeks on some risk biomarkers in obese older individuals. It was shown that plasma IL-6 level and TNF-α mRNA decreased significantly after the intervention (Lambert, Wright, Finck et al., 2008).

An increased sensitization of skeletal muscle to IL-6 at rest after exercise training may decrease IL-6 expression due to elevated basal IL-6R mRNA levels. Endurance training can increase muscle glycogen, in response to training, and it may decrease IL-6 production because there is an inverse relationship between muscle glycogen level and muscle-derived IL-6 production (Keller et al., 2005). However, some researchers suggested that exercise training-induced decrease in inflammatory biomarkers are associated with alteration of weight or body mass index (Aronson et al., 2004), and these variables did not significantly change in the CTG of the present study. Our findings suggest that 8 weeks of a combined training intervention in inactive young men has a positive influence on IL-6 as the cardiovascular diseases biomarker.

Daray et al. (2009) examined the effects of combined endurance and resistance training on C-reactive protein (CRP) levels in healthy adults (aged 18–24) and indicated that after 10 weeks of training, the plasma CRP concentration in the combined group decreased, although not significantly. These results are in line with our findings in the CTG. Martins et al. (2010) also reported that high-sensitivity CRP showed no significant change after 16 weeks of training in the resistance or aerobic groups. However, it substantially decreased in the two groups after 32 weeks of training. They concluded that short-term resistance or endurance training did not decrease the serum high-sensitivity CRP level. The lack of effects on CPR in the present study in the CTG might be linked to the slightly decreasing fat mass (%1.9) and BMI, as well as the short-term nature of the exercise programmes (Aronson et al., 2004; Rawson et al., 2003).
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The daily dietary and average caloric intake can affect the lipid profiles (Grundy & Denke, 1990). In the present study, the analysis of daily dietary data revealed no differences in the average caloric intake of the subject’s diets between the two periods or among the groups.

The results of the previous studies related to the effect of mixed training on the total cholesterol, triglyceride, LDL and HDL are inconsistent and limited. For example, LeMura et al. (2000) pointed out that the combined resistance and endurance training of 8 and 16 weeks respectively had not significantly improved total cholesterol, triglycerides, LDL and HDL in young women. Meanwhile, Sillanpää et al. (2009) showed that triglycerides and HDL did not significantly change in the combined training group and resistance training group after 10 and 21 weeks. On the other hand, some researchers revealed that the total cholesterol, and LDL had improved after mixed training (Park et al., 2003).

In the current study, after 8 weeks of the mixed training, no significant changes were observed in the total cholesterol, triglyceride and LDL, while HDL showed a significant increase in the CTG. Lack of improvements in the total cholesterol, triglycerides and LDL may be associated with the short duration of the training programme. However, these inconsistencies in the results of the studies could be attributed to factors such as the differences in the exercise training volume (caloric expenditure), type of exercise, duration of training, baseline serum lipid level, differences in daily dietary intake, baseline fitness level, and timing of blood sampling after intervention (Fletcher et al., 2001; Crouse et al., 1995).

The results of the analysis indicated that there was no significant change in the weight, BMI, as well as the waist and hip ratio for the CTG and the NTG, while reduction of the body fat percentage and an increase in skeletal muscle mass were shown to be significant after 8 weeks of the combined training. However, many studies have indicated that the combined training has a positive effects on the body composition, and that resistance training increases skeletal muscle mass (Tsuzuku et al., 2007), while aerobic training decreases body fat mass (McTiernan et al., 2007).

Some studies have shown an inverse relationship between aerobic capacity fitness and cardiovascular disease and VO$_2$max was identified as an independent predictor of cardiovascular events (Myers et al., 2002; Williams, 2001). The results of the current study also indicated that VO$_2$max had significantly increased (16.24%) and HR rest (-2.89%) had significantly decreased in the CTG. These findings showed that 8 weeks of the combined training could improve cardiovascular fitness in inactive young people. Furthermore, leg press and chest press strength also increased significantly after 8 weeks of the combined training. These findings are similar to those previously reported in the literature (Lambers et al., 2008; LeMura et al., 2000).

In conclusion, eight weeks of the combined training may be effective in improving some cardio risk factors, non-
traditional (Interlucine-6) or traditional (HDL), body composition (body fat percentage and muscle mass) and fitness (VO$_2$max and muscle strength) in inactive young men.

REFERENCES


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