The effect of concurrent training on glucose, lipid profile, liver enzymes, and lipid peroxidation in young men

El efecto del entrenamiento concurrente en la glucosa, perfil lipídico, enzimas hepáticas y la peroxidación lipídica en hombres jóvenes

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Abstract. The objective of this study was to determine the effect of concurrent training (CT) on glucose, liver function, lipid profile, and oxidative stress in young men. Sixteen moderately active young men (Age= 23.11 ± 4.20 yr., Height= 172.44 ± 6.50 cm, Weight= 73.52 ± 15.70 kg, BMI= 24.84 ± 5.85 kg/m²) trained for twelve weeks in separate in blocks of adaptation, development, and maintenance for four weeks each. Blood samples were collected at baseline and after CT to determine MDA glucose, TG, LDL-c, HDL-c, ALT, and AST. Repeated measures t-tests compared scores. Our results showed that no significant changes were observed in body weight (M= -0.14, p= 0.748, 95%CI= -0.79, 0.99), BMI (M= 0.04, p= 0.803, 95%CI= -0.27, 0.32), glucose (M= 0.88, p= 0.679, 95%CI= -2.87, 4.63), cholesterol (M= -0.19, p= 0.957, 95%CI= -7.00, 7.00), triglycerides (M= -9.00, p= 0.325, 95%CI= -25.19, 7.73), HDL-c (M= -0.60, p= 0.811, 95%CI= -6.52, 3.42), LDL-c (M= 2.20, p= 0.494, 95%CI= -4.20, 8.77), AST (M= -0.13, p= 0.954, 95%CI= -3.94, 3.00), ALT (M= 0.25, p= 0.923, 95%CI= -3.19, 4.44), VLDL (M= -1.80, p= 0.325, 95%CI= -5.04, 1.55), and MDA (M= 0.06, p= 0.621, 95%CI= -0.16, 0.29). The 40% of the participants responded positively to the CT program. Our findings revealed that a four-week CT program did not affect oxidative stress markers in young adults. Additionally, non-responders to the intervention should be carefully followed up to determine their personal threshold for improvement.

Keywords: Concurrent training, Oxidative stress, Lipid oxidation, Glucose, liver enzymes.

Resumen. El objetivo de este estudio fue determinar el efecto del entrenamiento concurrente (EC) en glucosa, función hepática, perfil lipídico y estrés oxidativo en hombres jóvenes. Dieciséis hombres jóvenes moderadamente activos (Edad= 23.11 ± 4.20 años, Altura= 172.44 ± 6.50 cm, Peso= 73.52 ± 15.70 kg, IMC= 24.84 ± 5.85 kg/cm²) entrenaron durante doce semanas en bloques separados de adaptación, desarrollo y mantenimiento, durante cuatro semanas cada uno. Se recolectaron muestras de sangre al inicio y después del EC para determinar MDA, glucosa, TG, LDL-c, HDL-c, ALT y AST. Se compararon las puntuaciones mediante pruebas t de medidas repetidas. Nuestros resultados mostraron que no se observaron cambios significativos en el peso corporal (M= -0.14, p= 0.748, IC del 95%: -0.79 y 0.99), IMC (M= 0.04, p= 0.803, IC del 95%: -0.27 y 0.32), glucosa (M= 0.88, p= 0.679, IC del 95%: -2.87 y 4.63), colesterol (M= -0.19, p= 0.957, IC del 95%: -7.00 y 7.00), triglicéridos (M= -9.00, p= 0.325, IC del 95%: -25.19 y 7.73), HDL-c (M= -0.60, p= 0.811, IC del 95%: -6.52 y 3.42), LDL-c (M= 2.20, p= 0.494, IC del 95%: -4.20 y 8.77), AST (M= -0.13, p= 0.954, IC del 95%: -3.94 y 3.00), ALT (M= 0.25, p= 0.923, IC del 95%: -3.19 y 4.44), VLDL (M= -1.80, p= 0.325, IC del 95%: -5.04 y 1.55) y MDA (M= 0.06, p= 0.621, IC del 95%: -0.16 y 0.29). El 40% de los participantes respondieron positivamente al programa de EC. Nuestros hallazgos revelaron que un programa de EC de cuatro semanas no afectó los marcadores de estrés oxidativo en adultos jóvenes. Además, los no respondedores a la intervención deben ser seguidos cuidadosamente para determinar su umbral personal de mejora.

Palabras clave: Entrenamiento concurrente, estrés oxidativo, glucosa, enzimas hepáticas.

Abbreviations: Physical activity (PA), body mass index (BMI), concurrent training (CT), maximum heart rate (MHR), endurance exercise (EE), endurance training (ET), Oxidative stress (OS), reactive oxygen species (ROS), malondialdehyde (MDA), triglycerides (TG), low-density lipoprotein cholesterol (LDL-c), high-density lipoprotein cholesterol (HDL-c), alanine aminotransferase (ALT) and aspartate aminotransferase (AST).

Introduction

An extensive body of evidence indicates that the lack of physical activity (PA) promotes adipose tissue accumulation, which potentially triggers systemic inflammation and increased oxidative stress (OS) (Sofra & Badami, 2020). The OS is defined as a condition in which the level of cellular oxidants (e.g., free radicals, reactive oxygen species -ROS-) produced exceeds the physiological capacity of neutralizing organs (i.e., endogenous antioxidant system) (Ceci et al., 2014). The ROS damage cell structures, nucleic acids, proteins, and lipids (Valko et al., 2006). Indeed, polyunsaturated fatty acid residues of phospholipids are susceptible to oxidation (Siems et al., 1995), allowing the production of peroxyl radicals (ROO•), which throughout a cyclization reaction produces endoperoxides, and, finally, malondialdehyde (MDA) (Valko et al., 2006). The MDA is a toxic molecule that induces mutagenic and cytotoxic processes (Voulgaridou et al., 2011). In addition, MDA is a relatively stable molecule and moves from its original location to other body fluids and can be used as a biomarker of OS (Gibala et al., 2012).

Regular PA elicits a wide range of health and well-being...
benefits, including improvement of aminotransferase and glucose levels, reduced insulin resistance, and improved fasting glucose levels (Kistler et al., 2011; Baba et al., 2006). Furthermore, regular mild - to moderate-intensity PA improves medical conditions related to OS-associated diseases (Gibala et al., 2012). In addition, it has been reported that repetitive muscular activity increases ROS formation by several pathways (e.g., mitochondria in the respiratory electron transport chain, NADPH oxidase) (Kawamura & Muraoka, 2018). Therefore, moderate-intensity PA promotes cells to adapt to increased ROS production and the consequent OS. The cell adaptation is achieved through the induction and expression of antioxidant enzymes by up-regulating protective systems like heat shock proteins and NF-kb. As a result, inflammatory mediator activity is inhibited (Teixeira de Lemos et al., 2012). Furthermore, muscle contractions also induce IL-6 release, which triggers the formation of anti-inflammatory cytokines (Rebillard et al., 2013). Therefore, the positive effect of PA depends on the training protocol since variations in intensity, duration, and type of exercises could activate different oxidant-antioxidant balance patterns leading to different cellular damage responses (Radak et al., 2013).

Concurrent training (CT) is an exercise modality that includes endurance and resistance exercises. A typical CT session includes various exercises performed in one session or on alternate days. Depending on the cardiorespiratory and muscular goals, the subjects can decide the sequence of endurance, muscle strength, or the combination of both modalities (Kang & Ratamess, 2014). CT positively affects body composition and the cardiovascular system more than training with either mode alone (Davis et al., 2008). Additionally, CT reduces oxidative damage by inhibiting protein oxidation; depending on the frequency, it can induce oxidative damage to lipids (Da Silva Medeiros et al., 2015). Therefore, given that the effect of CT on glucose, liver function, lipid profile, and oxidative stress in young men is still unclear, the present study aims to determine the responses of CT on these biochemical variables.

**Material and Methods**

**Subjects**

This study was performed with a sample size of no probabilistic 16 moderately-active young men volunteers (Age= 23.11 ± 4.20 yr., Height= 172.44 ± 6.50 cm, Weight= 73.52 ± 15.70 kg, BMI= 24.84 ± 5.85 kg/m²). Before undergoing the CT experimental sessions, the participants were given oral explanations about the assessment procedures, study objectives, possible benefits, and risks. Then, the participants read and signed an informed consent previously approved by the Research Ethics Committee of the State Cancer Institute of (the institution will be disclosed following peer review).

According to the medical information reviewed (PAR-Q & You), all participants were considered free from cardiovascular health problems. In addition, none complained of hypertension, diabetes, kidney disease, liver disease, and bone injuries and did not report supplement use in the past six months. Finally, none reported continuous exercise history.

**Study design**

The CT program consisted of three training blocks of four weeks each. The first stage was the adaptation phase, where participants trained for three days per week executing nine muscle strength exercises performed at 60% of one-repetition maximum (1-RM) combined with two days of running (EE) for 20-min at an intensity of 60-75% of the individual's maximum heart rate (MHR). On the third day, the participants completed a 15-min run, alternating efforts from 60% to 85% MHR. The second stage was the development phase, where participants trained four days per week. The stage consisted of 15 muscle strength exercises, six with weight, and a pyramidal method at an intensity of 75%, 80%, 85%, and 70% of 1-RM. The participants only used their own body weight for the remaining nine exercises. In addition, a combined session with two days of 25 min of EE at 60% to 75% MHR and two days of 15 min running endurance training (ET) alternating efforts at 60% to 85% MHR. Finally, the third stage was the maintenance phase, where participants trained five days per week. This stage included 11 muscle strength exercises, five with weight at 50% of 1-RM and four with explosive strength using arms and legs. A combined session with three days of 30 min of EE at 60% to 75% of MHR and two days of 15 min ET and alternating effort at 60% to 85% MHR was also performed.

**Blood sample and analysis**

For baseline blood sample collection, the subjects were instructed to avoid performing moderate or vigorous PA for at least two hours. At the end of the protocol, the participants did not perform activities before the blood samples were collected. For both data collection points, fasting blood samples (5 mL) were obtained from the cubital vein following standard procedures and after 8-h overnight fasting. The serum was separated and frozen at −80°C until further analysis. Glucose, triglycerides (TG), low-density lipoprotein cholesterol (LDL-c), high-density lipoprotein cholesterol (HDL-c), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) concentrations were measured using commercial assay kits (Biosystem Laboratories, Barcelona, Spain).

Lipid peroxidation was followed in the next way (Gambba-Gómez et al., 2016). In brief, serum samples (0.5 mL), a thioarbituric acid (TBA) (SIGMA Co., St. Louis, USA) solution (40.5 mL of 0.8% TBA, 40.5 mL of 20% acetic acid buffered at pH 3.5 with 1 M NaOH, 13.2 mL of 8.2% SDS, and 5.8 of distilled water) were added to each sample (4 mL) and
incubated at 96° C for 60 min. Then, the samples were cooled, mixed with n-butanol (5 mL), and centrifuged (3000 × g for 15 min). The absorbance was recorded at 532 nm using a spectrophotometer (Spectronic® 20 GenesysTM, Spectronic Instruments, United States). The results were expressed as micromoles of malondialdehyde (MDA) equivalents. For this, a calibration curve was prepared by diluting a standard solution of MDA (hydrolysis of tetraethoxypropane in 1% of sulfuric acid) (Mateos et al., 2005).

Transparency and Openness

According to the transparency and openness, we report how we determined our sample size, all manipulations, and all measures in this study.

All data, analysis code, and research materials are available with the first author of this research.

Statistical analysis

The data were analyzed using the IBM-SPSS software, v. 24 (IBM North America, New York, USA), and Excel (Microsoft Corp., Washington, USA). Descriptive and inferential statistics included mean and standard deviation (M±SD) and paired-sample t-test with robust bootstrap (1000 samples) 95% confidence intervals (CI95%) for the mean differences (i.e., baseline vs. after CT). Hedge’s g effect size and CI95% were computed and interpreted as small (0.20), medium (0.50), and large (0.80). Absolute reliability was also calculated using the typical error of measurement (TEM), coefficient of variability (CV), and the smallest worthwhile change (SWC) (Hopkins, 2000). Significance was set a priori at p < 0.05.

Results

The descriptive statistics of the biochemical parameters are shown in Table 1. The box-plots for glucose, total cholesterol, triglycerides, HDL-c, LDL-c, VLDL, AST, and ALT are shown in Figure 1. The lipid peroxidation scores of each participant are shown in Figure 2.

Table 1
Descriptive statistics for anthropometric and biochemical parameters (n =16). Values are M ± SD

<table>
<thead>
<tr>
<th>Variables</th>
<th>Baseline</th>
<th>Post-training</th>
<th>Hedge’s g effect size (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>70.9 ± 15.4</td>
<td>71.7 ± 14.9</td>
<td>-0.05 (-0.11, 0.00)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.1 ± 4.4</td>
<td>23.5 ± 4.4</td>
<td>-0.09 (-0.15, -0.03)</td>
</tr>
<tr>
<td>Glucose (mg·dL⁻¹)</td>
<td>95.3 ± 49.4</td>
<td>94.3 ± 40.2</td>
<td>-0.19 (-0.61, 0.22)</td>
</tr>
<tr>
<td>Triglycerides (mg·dL⁻¹)</td>
<td>152.5 ± 38.3</td>
<td>152.7 ± 36.1</td>
<td>-0.01 (-0.32, 0.30)</td>
</tr>
<tr>
<td>Cholesterol (mg·dL⁻¹)</td>
<td>73.5 ± 17.7</td>
<td>74.2 ± 16.1</td>
<td>-0.04 (-0.46, 0.38)</td>
</tr>
<tr>
<td>HDL-c (mg·dL⁻¹)</td>
<td>61.9 ± 32.0</td>
<td>59.7 ± 28.2</td>
<td>0.07 (-0.23, 0.38)</td>
</tr>
<tr>
<td>LDL-c (mg·dL⁻¹)</td>
<td>17.1 ± 9.8</td>
<td>18.9 ± 8.0</td>
<td>-0.19 (-0.59, 0.19)</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>29.8 ± 8.9</td>
<td>29.9 ± 6.1</td>
<td>-0.01 (-0.49, 0.46)</td>
</tr>
<tr>
<td>MDA (μmol·d⁻¹)</td>
<td>1.1 ± 0.4</td>
<td>1.0 ± 0.3</td>
<td>0.27 (0.34, 0.90)</td>
</tr>
</tbody>
</table>

Note: Hedge’s g effect size interpretation: 0.20= small, 0.50= medium, 0.80= large; BMI: Body mass index; HDL-c: High-density lipoprotein cholesterol; LDL-c: Low-density lipoprotein cholesterol; VLDL: Very low-density lipoprotein cholesterol; AST: aspartate aminotransferase; ALT: alanine aminotransferase; MDA: malondialdehyde.
No statistically significant differences were observed between baseline and post-training scores on body weight (M= -0.14, p= 0.748, 95%CI= -0.79, 0.99), BMI (M= 0.04, p= 0.803, 95%CI= -0.27, 0.32), glucose (M= 0.88, p= 0.679, 95%CI= -2.87, 4.63), cholesterol (M= -0.19, p= 0.957, 95%CI= -7.00, 7.00), triglycerides (M= -9.00, p= 0.325, 95%CI= -25.19, 7.73), HDL-c (M= -0.60, p= 0.811, 95%CI= -6.52, 3.42), LDL-c (M= -1.20, p= 0.494, 95%CI= -4.20, 8.77), AST (M= -0.13, p= 0.954, 95%CI= -3.94, 3.00), ALT (M= 0.25, p= 0.923, 95%CI= -3.19, 4.44), VLDL (M= -1.80, p= 0.325, 95%CI= -5.04, 1.55), and MDA (M= 0.06, p= 0.621, 95%CI= -0.16, 0.29). The effect size of the CT program was small for all variables. Indeed, the lower and upper limits CI95% always included zero (Table 1).

The CV for total cholesterol, TG, HDL-c, LDL-c, VLDL, AST, ALT, and MDA was high, whereas body weight, BMI, and glucose were small (i.e., < 10%) (Table 2). The SWC (original units) were computed based on the CV obtained from the variables. These represent the minimal meaningful change in the variable to determine the effectiveness of the exercise program. Based on the SWC, the proportion of participants who responded positively to the CT program, those who did not change, and those who did not respond in the theoretically expected direction are shown in figure 3. Overall, 40% of the participants responded to the CT program, and 60% either did not change (30%) or changed in the opposite expected direction (30%).
Mean variable change following concurrent training exercise, typical error of measurement, coefficient of variability (CV) and smallest worthwhile change (SWC)

| Variable          | Difference (Post-Pre) | TEM  | CV (%) | SWC
|-------------------|-----------------------|------|--------|------
| Weight (kg)       | -0.1                  | 1.3  | 1.8    | 0.4
| BMI (kg/m²)       | 0.0                   | 0.4  | 1.8    | 0.1
| Glucose (mg·dL⁻¹) | 0.1                   | 7.8  | 8.3    | 2.2
| Total cholesterol | -3.4                  | 17.8 | 11.4   | 5.0
| TG (mg·dL⁻¹)      | -12.6                 | 34.3 | 34.6   | 9.7
| HDL-c (mg·dL⁻¹)   | 1.0                   | 8.0  | 11.3   | 2.3
| LDL-c (mg·dL⁻¹)   | -1.8                  | 14.5 | 22.1   | 4.1
| VLDL (mg·dL⁻¹)    | -2.5                  | 6.9  | 34.6   | 1.9
| AST (U/L)         | -4.5                  | 15.8 | 49.4   | 4.5
| ALT (U/L)         | 1.2                   | 7.6  | 27.9   | 2.2
| MDA (μmol·dL⁻¹)   | -0.1                  | 0.3  | 30.5   | 0.1

Note: BMI: Body mass index; HDL-c: High-density lipoprotein cholesterol; LDL-c: Low-density lipoprotein cholesterol; VLDL: Very low-density lipoprotein cholesterol; AST: aspartate aminotransferase; ALT: alanine aminotransferase; MDA: malondialdehyde.

**Discussion**

This research aimed to evaluate the responses of CT to oxidative stress in young men. The main finding was that a CT program consisting of three training blocks of four weeks each did not significantly change glucose level, lipid profile, AST, ALT levels, and oxidative stress (i.e., serum lipid oxidation). The effect size of the CT program was small to elicit significant changes in the variables studied.

The evidence suggests that CT could be an alternative to improve lipid profiles and glucose levels. Studies reported significant improvements in lipid profile parameters and glucose levels following eight weeks of CT (Arazi et al., 2013; Ghahramanloo et al., 2009). However, in the present study, no significant changes were found. We attributed this disagreement to the differences in the program length between CT programs and because we did not control the participant’s diets. In addition, it also could be related to the liver enzyme results, where a positive effect is observed after eight weeks of CT (Salehani & Alizadeh, 2019).

Previous studies suggest that ROS are generated during exercise, modulating signaling pathways and the level of muscle contraction. Thus, low ROS levels stimulate force production, whereas high levels attenuate it (Radák et al., 2008). Consequently, the association between OS and PA is related to the specific characteristic of the PA prescription. The intense PA could increase OS and allows endurance exercise-induced lipid peroxidation (Mastaloudis et al., 2001) and the daily increased intensity of PA (i.e., resistance training with weights from light to moderately severe) decreases the lipid peroxidation (Zabet et al., 2019) has been reported that the CT programs, evaluated OS markers (i.e., protein and lipid oxidation) in obese individuals performing two types of CT with a frequency of 3 and 5 days/week at the same intensity, respectively. The results showed that less frequent exercise decreased protein damage, and high-frequency exercise increased lipid peroxidation (i.e., MDA levels) (Da Silva Medeiros et al., 2015). However, we did not observe a difference in lipid peroxidation despite increased frequency through the CT period. This could be due to the difference between study designs and the subject’s characteristics. Therefore, more studies are needed to elucidate the mechanisms involved in the CT exercise.

A physical exercise regime consisting of CT demands a specific program according to the person’s goals. Specifically, the sports training theory describes that physical exercise needs to be comprised of a particular program based on the general adaptation syndrome described by Seyle (Moreno & Ordoño, 2009; Ocaña, 1998). That theory proposes that the body undergoes a series of physiological responses in three stages when exposed to stress. The first stage is the alarm, where the body mobilizes its resources to respond to the stressor. The second stage is resistance, where the body adapts and copes with the stressor. Finally, the third stage is exhaustion, where the body’s resources deplete, leading to physical and emotional exhaustion. According to these principles, the sports training theory describes that when the subject takes time for active rest on the same size of the stimulus (exercise), like during a CT program, the performance level increase, and also the muscle mass, energy, ventilatory capacity, maximum strength, stretching, and heart capacity, among others. Therefore, the adaptation syndrome following the training blocks might be the reason for the lack of statistical significance in the present study after 12 weeks of training; in other words, the stimuli provided by the CT program to 60% of the participants were not strong enough to elicit biological adaptations. Yet, for 40% of the participants, meaningful individual changes in the biochemical variables were observed (figure 3).

**Conclusions**

The present study’s data suggest that four weeks of a CT program does not affect oxidative stress markers (serum lipid...
oxidation). Non-responders to the intervention must be carefully followed-up to determine their personal threshold for improvement. Prospective studies are needed, with increased CT program time recommended to elucidate the possible effect on these biochemical parameters.

Acknowledgements

The research presented in this project was supported by Movement is Health Foundation. The authors declare no conflicts of interest.

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