



## Acute effect of concurrent training on hematological and hepatic profile, and muscle injury markers in trained individuals

*Efecto agudo del entrenamiento concurrente en el perfil hematológico y hepático, y en los marcadores de daño muscular en individuos entrenados.*

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### Abstract

**Objective:** to analyze the acute effect of concurrent training on hematological and hepatic profile, and muscle injury markers in trained individuals.

**Methodology:** 11 trained males ( $27.0 \pm 1.13$  years old) were undergone to body composition, muscle strength, cardiorespiratory fitness evaluation and a concurrent training (CT) session. Blood samples of glucose (GLU), total cholesterol (TC), triglycerides (TG), HDL-cholesterol fraction (HDL), LDL-cholesterol fraction (LDL), gamma glutamyl transferase (GGT), alanine amino transferase (ALT), aspartate amino transferase (AST), urea (Ur), bilirubin (Bil), total protein (TP), lactate (LAC), Creatine kinase (CPK) and lactate dehydrogenase (LDH) were collected before and immediately after the CT.

**Results:** paired Student's t-test showed a significant increase on HDL ( $p=0.037$ ), GGT ( $0.015$ ), AST ( $p=0.0001$ ), Ur ( $p=0.002$ ), Bil ( $p=0.0001$ ), TP ( $p=0.024$ ), LAC ( $p=0.015$ ), CPK ( $p=0.0001$ ) and LHD ( $p=0.005$ ) levels.

**Conclusions:** A single session of concurrent training increased the blood concentration of the biochemical parameters studied related to cardiometabolic health in the evaluated individuals. Furthermore, it seems that the individuals' fitness level may influence the behavior of biochemical variables in response to physical exercise.

### Keywords

Training, concurrent training, muscle injury, hematological profile, hepatic profile.

### Resumen

**Objetivo:** analizar el efecto agudo del entrenamiento concurrente sobre el perfil hematológico, hepático y los marcadores de lesión muscular en individuos entrenados.

**Metodología:** 11 varones entrenados ( $27,0 \pm 1,13$  años) fueron sometidos a evaluación de la composición corporal, de la fuerza muscular, de la aptitud cardiorrespiratoria y una sesión de entrenamiento concurrente (CT). Muestras de sangre de glucosa (GLU), colesterol total (TC), triglicéridos (TG), fracción de colesterol HDL (HDL), fracción de colesterol LDL (LDL), gamma glutamil transferasa (GGT), alanina amino transferasa (ALT), aspartato se recogieron aminotransferasa (AST), urea (Ur), bilirrubina (Bil), proteína total (TP), lactato (LAC), creatina quinasa (CPK) y lactato deshidrogenasa (LDH) antes e inmediatamente después de la TC.

**Resultados:** la prueba t de Student pareada mostró un aumento significativo en los niveles de HDL ( $p=0,037$ ), GGT ( $0,015$ ), AST ( $p=0,0001$ ), Ur ( $p=0,002$ ), Bil ( $p=0,0001$ ), TP ( $p=0,024$ ), LAC ( $p=0,015$ ), CPK ( $p=0,0001$ ) y LHD ( $p=0,005$ ).

**Conclusiones:** Una sola sesión de entrenamiento concurrente aumentó la concentración sanguínea de los parámetros bioquímicos estudiados relacionados con la salud cardiometabólica en los individuos evaluados. Además, parece que el nivel de condición física de los individuos puede influir en el comportamiento de las variables bioquímicas en respuesta al ejercicio físico.

### Palabras clave

Entrenamiento, Entrenamiento concurrente, lesión muscular, perfil hematológico, perfil hepático.

## Introduction

Sedentary behavior and physical inactivity are among the main modifiable risk factors for cardiovascular diseases and all-cause mortality worldwide (Lavie et al., 2019). To reduce sedentary demeanor, lifestyle changes and increased physical activity levels are strongly suggested (Lachman et al., 2018; Mello et al., 2019). The relative risks associated with sedentary time are higher among individuals who are not regularly physically active (Lavie et al., 2019).

Regular physical activity is crucial for reducing cardiovascular morbidity and mortality rates (Yavari et al., 2012). Furthermore, it can help improve body composition and weight control (Leońska-Duniec et al., 2019), and positively affect biochemical parameters related to chronic inflammation, stress, and cardiometabolic risk (Rosa & Mello, 2016; Figueira et al., 2019; Baptista, Machado-Rodrigues & Martins, 2018).

Training programs incorporating varied exercise modalities have been shown to reduce cardiometabolic risk factors and positively influence biophysical and biochemical health variables (Monteiro-Lago et al., 2019; Johnson et al., 2019). However, information is scarce regarding the timing of observable health benefits following the implementation of a training program and the magnitude of the effects of different exercise aspects on these benefits.

Information on the gains from exercise in sedentary or untrained individuals is well-documented (Johnson et al., 2019; Ramírez-Vélez et al., 2019). Despite previous adaptations, trained individuals still exhibit acute physiological and biochemical responses to new training stimulus, supporting the relevance of investigating their short-term effects (Mathunjwa et al., 2021). Yet, the extent to which previously trained individuals can continue to benefit from exercise remains unclear and requires further investigation.

Therefore, the aim of this study was to analyze the acute effect of concurrent training session on hematological and hepatic profile, and muscle injury markers in trained individuals.

## Method

### Study design

Experimental research (Thomas, Nelson & Silverman, 2010).

### Sample

It was composed by 11 male individuals, aged  $27.0 \pm 1.13$  yrs., regular practitioners of physical exercise during six months, three times per week. Inclusion criteria: participants did not perform any type of physical exercise for at least the 36 hours that preceded them. Exclusion criteria who used some medicine or substance who could influence the blood sample; who lost one of the data collection moments; who presents pathology or muscle articular injuries, or others clinic scream that could influence the results and performance.

### Ethics

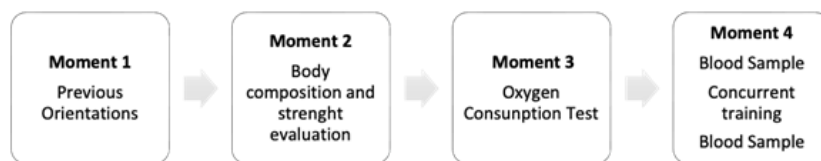
The participants signed an informed consent document to participate in research involving human subjects in accordance to the Declaration of Helsinki (WMA, 2008). The research project was also approved by the Ethics Committee in Research Involving Human Beings of the Universidade Federal do Estado do Rio de Janeiro under protocol number CAAE: 40642114.9.0000.5285.

### Data collection

Data collection was carried out in four different moments with an interval of 48 hours between them as present at figure 1.



Figure 1. Data collection design



### ***Previous orientations***

At this phase, the informed consent document was applied and also was explained how data collection would be carried out and orientations were presented for each of the different moments of the study: the assessment of body composition, muscle strength and oxygen consumption evaluation, in addition to the collection of blood samples and intervention.

To the body composition assessment, muscle strength and cardiorespiratory fitness evaluation, volunteers were oriented to did not perform any kind of physical exercise 8 hours before this stage. For the moment 4 a 12-hour fast was recommended.

### ***Body composition***

Measurements of total body mass, height, body mass index (BMI), body fatness (%F), fat mass (FM), and lean body mass (LBM) were performed according to International Society for the Advancement of Kinanthropometry – ISAK protocol (Marfell-Jones, Stewart & Carter, 2006). Three skinfolds protocol for male (pectoral, abdominal and thigh) was used (Jagim et al. 2023), in association to the equation by Jackson; Pollock (Jackson & Pollock, 2007).

### ***Cardiorespiratory Fitness***

The Cooper test was used to assess the levels of cardiorespiratory fitness (VO<sub>2</sub> maximum) (Bandyopadhyay, 2014; ACSM, 2013) on a 400-meter athletics track regulated by the IAAF (International Athletics Federation) at the Army Physical Education School (EsEFEx), Rio de Janeiro, Brazil.

### ***Muscle Strength***

Individuals were submitted to 1 maximum repetition (1RM) test in the TechnoGym® equipment: Supported Rowing, Leg Press 45°, Horizontal Supine, Extension Chair, Elbow flexion and Flexor table following the guidelines proposed by Beachle; Earle (Baechle & Earle, 2016) to determine training intensity.

### ***Biochemistry markers***

Biochemical variables were performed by analysis of blood samples, after a 12-hour fasting, using the colorimetric method, BT 3000 plus, Wiener Lab®. Blood sample for hematological and hepatic profile, and muscle injury markers were performed before and immediately after the exercise intervention. In each of them, approximately 3mL of blood was withdrawn from each participant, totaling approximately 12mL at the end of the study. After blood sample analysis procedures, leftover laboratory samples containing blood were discarded in accordance with ANVISA Legislation - RDC 306 of December 7, 2004, which provides for the technical regulation for waste management of health services. All tests were duplicated, and the coefficients of variation (CV) obtained were less than 3% (Friedewald, Levy & Fredrickson, 1972).

-Hematological profile: glucose, (GLU) total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL) and low-density lipoprotein (LDL).

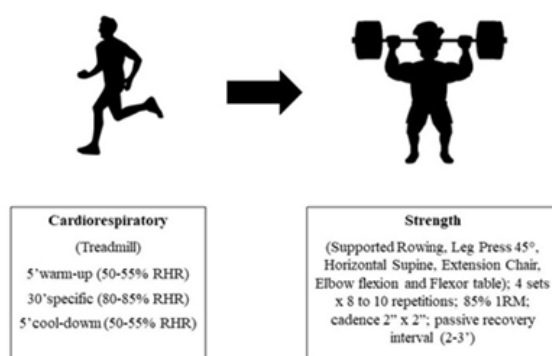
-Hepatic profile: aspartate aminotransferase (AST), alanine aminotransferase (ALT) Gamma glutamyl transpeptidase (GGT), urea (Ur), bilirubin (Bil), and total protein (TP).

-Muscle injury markers: lactate (LAC), lactate dehydrogenase (LDH) and creatine kinase (CPK).

## Intervention

- Breakfast: after blood sample, participants had a standard breakfast meal consisting of 200 ml of 0% fat yogurt, two slices of light whole-wheat bread, 30g of fresh cheese, 10g of vegetable margarine, 200 ml of orange juice and 1 medium banana prescribed by a nutritionist.
- Concurrent Training: started 40 minutes after breakfast. The session was composed by cardiorespiratory training (running on a treadmill) and strength training on equipment.

Figure 2. concurrent training. legend: RHR (heart rate reserve); 1RM (one maximum repetition test); cadence 2" x 2" (execution speed of approximately four seconds).



## Data analysis

The statistical procedures were processed with the Statistical Package for the Social Sciences software (SPSS Statistics 25, Chicago, USA). Descriptive statistics were applied to determine the mean, standard deviation, minimum and maximum values. The Shapiro-Wilk and Levene's test was used to verify normality and homogeneity of data. Paired Student's t-test was used to analyze the intragroup comparisons. The effect size of Cohen (d) was measured to analyze the magnitude of a treatment effect. It was classified into small ( $d \leq 0.2$ ); medium ( $0.2 < d < 0.79$ ) and large ( $d \geq 0.8$ ) (Cohen, 1988). Significant level was  $p < 0.05$ .

## Results

The characteristics of body composition, cardiorespiratory fitness and normality of the sample are presented in table 1. It was observed a normal distribution for the analyzed variables.

Table 1. Anthropometric characteristics and cardiorespiratory fitness.

	Mean	sd	Minimum	Maximum	SW (p-value)
Age (years)	27	1.13	25	29	0.55
BM (kg)	74.53	5.39	67.45	84.85	0.25
Height (m)	1.76	0.07	1.62	1.84	0.39
Fat%	8.96	2.66	5.60	13.64	0.26
BFM	6.70	2.14	3.87	11.26	0.36
LBM	67.72	5.17	60.28	69.36	0.54
VO2máx	58.61	3.63	51.02	62.47	0.92

Legend: BM: body mass; BMI: body mass index; Fat%: percentage of body fat; BFM: body fat mass; LBM: lean body mass; VO2máx: Maximum Oxygen Consumption in ml/Kg/min; sd: standard deviation; SW: Shapiro-Wilk normality test.

Table 2 presents the values (mean  $\pm$  standard deviation) referring to intragroup comparisons of hematological profile in the moments before (Pre) and immediately after the concurrent training session (Post).

Table 2. Hematological profile.

	Concurrent Training				
	Pre	Post	<i>p-value</i>	$\Delta\%$	<i>d</i>
GLU (mg/dL)	81,36 $\pm$ 8,49	91,55 $\pm$ 18,12	0,075	12,5	1,20
TC (mg/dL)	156,91 $\pm$ 25,79	165,73 $\pm$ 23,16	0,609	5,6	0,34
TG (mg/dL)	81,36 $\pm$ 24,63	85,36 $\pm$ 32,04	0,147	4,9	0,16
HDL (mg/dL)	37,45 $\pm$ 6,55	47,73 $\pm$ 11,23	0,037*	27,4	1,57
LDL (mg/dL)	103,18 $\pm$ 25,71	101,00 $\pm$ 21,33	0,149	-2,1	-0,08

Legend: GLU: glucose (mg/dL); TC: total cholesterol (mg/dL); TG: triglycerides (mg/dL); HDL: HDL-cholesterol fraction (mg/dL); LDL: LDL-cholesterol fraction (mg/dL); \*: significant difference ( $p < 0.05$ ); *d*: effect size using Cohen's *d* index.

Table 3 and 4 present hepatic profile and muscle injury markers post concurrent training session, and it was observed that there was a significant increase ( $p < 0.05$ ) GGT and AST levels. LAC, CPK and LDH also showed significant increases ( $p < 0.05$ ) after the intervention.

Table 3. Hepatic profile.

	Concurrent Training				
	Pre	Post	<i>p-value</i>	$\Delta\%$	<i>d</i>
GGT (U/L)	19,82 $\pm$ 8,52	24,73 $\pm$ 9,80	0,015*	24,8	0,58
ALT (U/L)	20,91 $\pm$ 9,01	19,36 $\pm$ 12,70	0,232	-7,4	-0,17
AST (U/L)	26,91 $\pm$ 5,39	28,36 $\pm$ 7,70	0,0001*	5,4	0,27
Ur (mg/dL)	35,00 $\pm$ 5,71	38,27 $\pm$ 5,41	0,002*	9,3	0,22
Bil (mg/dL)	0,58 $\pm$ 0,39	0,62 $\pm$ 0,45	0,0001*	6,8	0,09
TP (g/dL)	7,25 $\pm$ 0,37	7,70 $\pm$ 0,73	0,024*	6,2	0,28

Legend: GGT: gamma glutamyl transferase; ALT: alanine amino transferase; AST: aspartate amino transferase; Ur: urea; Bil: bilirubin; TP: total protein; \*: significant difference ( $p < 0.05$ ); *d*: effect size using Cohen's *d* index.

Table 4. Muscle Injury Markers.

	Concurrent Training				
	Pre	Post	<i>p-value</i>	$\Delta\%$	<i>d</i>
LAC (mmol/L)	8,65 $\pm$ 1,97	29,84 $\pm$ 13,27	0,015*	245,0	10,75
CPK (U/L)	352,09 $\pm$ 234,14	431,09 $\pm$ 261,93	0,0001*	22,4	0,34
LDH (U/L)	306,36 $\pm$ 24,92	331,36 $\pm$ 28,68	0,005*	8,2	1,00

Legend: LAC: lactate; CPK: Creatine kinase; LDH: lactate dehydrogenase; \*: significant difference ( $p < 0.05$ ); *d*: effect size using Cohen's *d* index.

## Discussion

This study aimed to evaluate the behavior of biochemical parameters related to cardiometabolic health in response to concurrent training. Preliminary evaluation of the variables indicated that the study participants had adequate levels in most biochemical parameters, except for HDL, most likely due to their good physical conditioning and body composition. For HDL to be considered adequate, its concentration should be above 40 mg/dL, a level not achieved by the volunteers, which can be explained by nutritional factors.

The concurrent training session increased all analyzed variables. This can be explained by the energy production for muscle contraction, which is achieved through the degradation of macronutrients that are re-synthesized by metabolic pathways predominant during exercise. The increase in blood GLU levels is expected, as indicates an availability for subsequent cellular use. The acute increase in LAC levels and the high effect size observed for this variable can be attributed to the lactate energy production pathway, influenced by the volume and intensity of the exercise session (Hargreaves & Spriet, 2020). Findings from the study by Markov et al. (2025) involving young female judo athletes demonstrated acute elevations in LAC and GLU levels following concurrent training sessions, with responses modulated by the order of exercise. Although the protocol and population assessed differ from the present study, these results support the metabolic effects observed here, reinforcing that combining strength and endurance stimuli in a single session can induce significant changes in markers related to energy metabolism, regardless of sample characteristics.

In the present study, an increase in the concentrations of blood lipid profile variables such as TC, TG, HDL, LDL was observed, and can improved by physical exercise (Wilund et al., 2009). They found a sig-



nificant reduction in TC, TG, and LDL levels, with an increase in HDL, in individuals with high cardiometabolic risk after six months of cardiorespiratory exercise performed three times a week at an intensity between 50% and 70% of reserve heart rate. However, our findings occurred after a single session of concurrent training (Wilund et al., 2009).

Cho et al. (2019) also demonstrated that physical exercise can improve lipid metabolism. After eight weeks of concurrent training, they observed a significant reduction in TG and an increase in HDL levels, regardless of dietary intervention. These findings differ from the present study, which also included an intervention based on concurrent training but was conducted in a single session (Cho et al., 2019).

Schroeder et al. (2019) claimed that concurrent training offers more benefits to cardiovascular health parameters compared to aerobic or resistance exercises performed in isolation. They observed a reduction in GLU, TC, HDL, and LDL levels after eight weeks of concurrent training, which contrasts with the results of the present study. Variations in sample characteristics, volunteer fitness levels, and intervention duration might explain the discrepancies between the findings (Schroeder et al., 2019).

Supporting these results, Jurasz et al. (2022) also observed an acute increase in LAC concentrations across the different groups in their sample of trained individuals. However, unlike the concurrent training protocol, their intervention involved incremental exercise performed on a cycle ergometer with a workload reaching 200W (Jurasz et al., 2022).

Antunes et al. (2020) showed that blood concentrations of TC, LDL, and HDL in response to acute exercise can vary based on the participants' physical fitness level and the intensity of the training session. They found that only TG levels decreased at all exercise intensities, both in individuals with low VO<sub>2</sub>max and those with high VO<sub>2</sub>max. In the present study, which included trained individuals, there was a significant increase in all analyzed biochemical parameters (Antunes et al., 2020).

One hypothesis that might explain the results is the duration of the intervention. Yavari et al. (2012) observed significant reductions in GLU, TC, TG, LDL, and HDL parameters in the group that practiced concurrent training three times per week for one year. Additionally, the participants in their study had type II diabetes, while those in our study did not (Yavari et al., 2012).

Amaro-Gahete et al. (2021) observed a reduction in blood levels of GLU, TC, TG, and HDL, differing from the findings of our study. Their concurrent training intervention lasted 12 weeks, while the intervention in this research consisted of a single session alone. Furthermore, their sample included men with obesity, while the sample in this study consisted of men with adequate body composition and high physical fitness, which could explain the discrepancy between the results (Amaro-Gahete et al., 2021).

The hepatic profile data showed significant increases in GGT ( $p=0.015$ ,  $\Delta\%=24.8$ ), AST ( $p=0.0001$ ,  $\Delta\%=5.4$ ), Ur ( $p=0.002$ ,  $\Delta\%=9.3$ ), Bil ( $p=0.0001$ ,  $\Delta\%=6.8$ ) and TP ( $p=0.024$ ,  $\Delta\%=6.2$ ). These changes suggest enhanced hepatic enzyme activity, increased protein catabolism, and anabolic response due to the physiological adaptations to concurrent training. Alanine aminotransferase (ALT) did not show significant change, indicating no severe hepatocellular damage. Muscle injury markers data reveal significant increases in LAC ( $p=0.015$ ,  $\Delta\%=245.0$ ), CPK ( $p=0.0001$ ,  $\Delta\%=22.4$ ) and LDH ( $p=0.005$ ,  $\Delta\%=8.2$ ) that indicate increased muscle stress and adaptation due to the high-intensity exercise components of the CT program, reflecting muscle remodeling and metabolic stress.

Effects of different sequences in concurrent strength and endurance training on performance, highlighting that while endurance improvements are largely unaffected by the training order, the strength adaptations are significantly influenced by it. Specifically, the endurance-strength sequence promotes muscle hypertrophy through better skeletal muscle protein synthesis, whereas the strength-endurance sequence enhances neuromuscular efficiency, relative strength, and explosive power. Neuromuscular adaptations benefit more when strength training precedes endurance activities, optimizing motor unit recruitment and neural drive. Molecularly, strength-first training activates mTOR pathways more effectively, while endurance-first activates AMPK, which can inhibit mTOR signaling if not adequately spaced. Practical recommendations suggest tailoring the training sequence to individual goals, with a 3-hour gap between sessions to minimize interference for optimal results (Zhang et al., 2023).

Egan and Sharples (2023) emphasizes that a deeper understanding of molecular adaptations can refine exercise prescriptions tailored to individual needs, optimizing performance and health outcomes. Their review explores how different types of exercise -namely aerobic, resistance, and high-intensity interval



training (HIIT) - activate specific molecular pathways that drive skeletal muscle adaptations. Acute exercise triggers pathways such as AMPK, mTOR, and MAPK, which regulate transcription, protein synthesis, and muscle remodeling. Aerobic exercise enhances oxidative capacity and mitochondrial biogenesis, resistance exercise promotes hypertrophy and strength, and HIIT combines the benefits of both. Exercise also induces epigenetic changes and balances muscle protein synthesis and degradation through mTORC1 signaling and proteasome systems. These responses are based on fundamental physiological principles and occur regardless of training status, although the magnitude of response and recovery may differ between trained and sedentary individuals, as sedentary subjects often show delayed or attenuated recovery due to lower baseline fitness (Egan & Sharples, 2023).

Long-term high-level exercise training leads to distinct molecular adaptations in skeletal muscle. Multi-omics analysis revealed significant differences in fatty- and amino acid metabolism and transcription factors between endurance and strength athletes versus controls. Endurance athletes had higher carnitine derivatives, while strength athletes showed more phospholipid metabolites. Acute resistance exercise induced more gene expression changes than endurance exercise, particularly down-regulating mitochondrial and ribosomal pathways in endurance athletes. These results highlight a "transcriptional specialization effect" and provide insights into the molecular basis of training adaptations (Reitzner et al., 2023).

## Conclusions

A single session of concurrent training increased the blood concentration of the studied biochemical parameters related to cardiometabolic health in the evaluated individuals.

This acute effect of the performed training highlights the necessity for continuous training for physiological adaptations to occur in response to exercise (chronic adaptations). This continuity allows the reduction, stabilization, and control of the levels of the analyzed biochemical variables to be observed. Furthermore, it seems that the individuals' fitness level may influence the behavior of biochemical variables in response to physical exercise.

Future studies should evaluate the effects of a longer-duration concurrent training program and/or different volume and intensity characteristics on the levels of biochemical parameters related to cardiometabolic health in individuals with varying physical fitness levels.

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## Declaration of interest statement

All authors declare that they have no conflicts of interest with any public or private financial entity in relation to the content of this manuscript.

## Author's contribution

All authors contributed equally to the research process and the writing of the manuscript.



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