
ACUTE EFFECTS OF PHYSICAL EXERCISE AT DIFFERENT INTENSITIES ON INFLAMMATORY MARKERS IN OBESE ADOLESCENTS**EFEITO AGUDO DO EXERCÍCIO FÍSICO EM DIFERENTES INTENSIDADES SOBRE OS MARCADORES INFLAMATÓRIOS EM ADOLESCENTES OBESOS****Géssika Castilho dos Santos¹, Wayne Ferreira Faria¹, Jeffer Eidi Sasaki², Rui Marques Gonçalves Elias¹ e Antonio Stabelini Neto¹**¹Universidade Estadual do Norte do Paraná, Jacarezinho-PR, Brasil.
²University of Alabama at Birmingham, Birmingham-AL, United States.**ABSTRACT**

Obesity promotes a state of chronic low-grade inflammation of white adipose tissue, which is associated with different chronic diseases. While exercise induces an anti-inflammatory environment, little is known about the acute effects of continuous exercise at different intensities on inflammatory markers in obese adolescents. This study included 10 obese adolescents of both sexes, between 15 and 18 years of age. Volunteers performed two 30-minute exercise sessions. The 1st exercise session was performed at moderate intensity (55% VO_{2peak}) and the 2nd at vigorous intensity (75% VO_{2peak}), with a one-week interval between sessions. Blood samples were collected 10 minutes before and 20 minutes after the exercise sessions. Inflammatory markers were analyzed using the chemiluminescence method. Statistical analysis was performed using the two-factors ANOVA test for repeated measures and statistical significance was set at 5% (p < 0.05). The results showed that 30 minutes of moderate aerobic exercise resulted in significant changes in circulating levels of TNF- α (6.23 \pm 1.64 to 7.03 \pm 1.49) and IL-6 (3.35 \pm 1.53 to 3.85 \pm 1.73). The results of this study showed that a single exercise session of moderate intensity lasting for 30 minutes induced acute responses in inflammatory markers in the obese adolescents evaluated.

Keywords: Physical activity; Inflammatory cytokines; Obesity.**RESUMO**

A obesidade promove um estado de inflamação crônica de baixo grau do tecido adiposo branco, o qual está associado a doenças crônicas. O exercício físico induz um ambiente anti-inflamatório, porém pouco se sabe sobre o efeito agudo do exercício físico contínuo em diferentes intensidades sobre os marcadores inflamatórios em adolescentes obesos. Participaram deste estudo 10 adolescentes obesos de ambos os sexos, entre 15 e 18 anos. Os voluntários realizaram duas sessões de 30 minutos de exercício, sendo a 1ª sessão na intensidade moderada (55% VO_{2pico}) e a 2ª sessão na intensidade vigorosa (75% VO_{2pico}), com o intervalo de uma semana entre cada sessão. As amostras sanguíneas foram coletadas 10 minutos pré e 20 minutos pós exercício. Os marcadores inflamatórios foram analisados através do método Quimiluminescência. Para análise estatística foi utilizado o teste ANOVA para medidas repetidas com dois fatores e o nível de significância adotado foi de 5% (p < 0,05). Os resultados indicaram que 30 minutos de exercício aeróbico moderado resultou em mudanças significativas nos níveis circulatórios de TNF- α (6,23 \pm 1,64 para 7,03 \pm 1,49) e IL-6 (3,35 \pm 1,53 para 3,85 \pm 1,73). A partir dos resultados encontrados no presente estudo, pode-se concluir que uma única sessão de exercício físico de intensidade moderada com 30 minutos de duração induziu respostas agudas nos marcadores inflamatórios nestes adolescentes obesos.

Palavras-chave: Atividade física; Citocinas inflamatórias; Obesidade.**Introducion**

Obesity prevalence has increased substantially, becoming a global epidemic that affects both developed and developing countries¹. Obesity is a chronic disease of multifactorial etiology which can be characterized by excessive accumulation of body fat, representing a risk to health².

One of the main concerns related to obesity is the presence of a state of chronic low-grade inflammation in white adipose tissue. This adipose tissue, in particular the visceral type, synthesizes and secretes biologically active substances. When secreted in an unregulated manner, these substances can induce a chronic state of systemic low-grade inflammation^{3,4},

strongly associated with co-morbidities such as insulin resistance, type 2 diabetes, atherosclerosis, and heart disease, and can contribute to metabolic dysregulation⁵.

Inflammation is characterized by the elevation of inflammatory markers and cytokines. Evidence suggests that adipose tissue in excess results in increased levels of tumor necrosis factor (TNF- α), interleukin 6 (IL-6), leucocytes, and C-reactive protein (CRP), contributing to insulin resistance and endothelial dysfunction. Thus inflammatory condition may be reversible with weight reduction and/or regular physical exercise practice, which has been shown to modulate immune function and anti-inflammatory effects^{6,7}.

After a single exercise session, cytokines are produced and released, inducing an inflammatory response⁸. However, when regular exercise takes place, production of anti-inflammatory cytokines by neutralizing pro-inflammatory cytokines is increased, introducing a positive anti-inflammatory environment. Pedersen⁹ reported increased concentrations of IL-6 post-exercise, and this cytokine stimulated the appearance of other anti-inflammatory cytokines, including IL-10, IL-1ra, and soluble TNF- α receptors¹⁰.

Although studies have investigated changes in the post-exercise circulatory concentrations of these cytokines¹¹, investigations involving obese adolescents are still scarce. Thus, to understand the acute effects that physical exercise have on the modulation of the immune system, it is necessary to explore how different physical exercise intensities stimulate these inflammatory markers in obese adolescents. In this sense, the objective of the present study was to analyze the acute effects of physical exercise at different intensities on inflammatory markers in obese adolescents.

Methods

Participants

First, the researcher contacted each institution in the city of Jacarezinho, State of Paraná, to explain the research and to verify if the school obtained the target population of the study and interest in participating. Subsequently a private conversation was held with each student to invite him / her to participate in the research.

Ten obese adolescents of both sexes volunteered for this study. Participants were recruited in schools and were required to meet the following inclusion criteria: aged between 15 and 18 and classified as obese according to the reference curves of the Centers for Diseases Control and Prevention (CDC)⁶, BMI \geq 95th percentile. The exclusion criteria were: continuous use of medication; not having participated in all stages of data collecting; having any kind of genetic, neoplastic or mental disease that could interfere with the understanding of the activities or performance during the study. Volunteers who agreed to participate received the "informed consent form", which was completed and signed by themselves and by their responsible. Table 1 presents participant characteristics.

Experimental Design

This study was approved by the Human Research Ethics Committee of the State University of Londrina (UEL) (ruling number: 1.077.560). Study participants attended the Human Movement Biodynamic Laboratory at the Health Sciences Center at the State University of Northern Parana (UENP) on three occasions, with a one-week interval between each visit.

Visit 1: Volunteers returned the informed consent form signed by his (her) guardian and also signed the informed assent form. During this visit, anthropometric measurements (weight, height and waist circumference) were taken and maturation was evaluated based on the Tanner stages. Participants then underwent an incremental test on a treadmill and physical activity level was assessed using a pedometer for 7 (seven) consecutive days.

Visits 2 and 3: Participants were instructed to refrain from any kind of physical exercise for at least 48 hours prior to each session and maintain their nutrition and hydration routines during the study. The protocol consisted of: 1) completing a questionnaire on stress level¹²; 2) blood collecting before starting the activity; 3) 30 minutes of exercise on a treadmill; and 4) post-exercise blood collecting. The exercise intensity on the second visit was 55% of VO_{2peak} (moderate intensity exercise) and on the third visit 75% of VO_{2peak} (vigorous intensity exercise).

Anthropometry

Height and weight were measured to nearest 0.1 cm and 0.1 kg using a fixed vertical stadiometer and a digital scale, respectively. Body mass index (BMI) was calculated dividing body weight (kg) by squared height (m^2). Adolescents were classified as obese if BMI values $\geq 95^{th}$ percentile, in accordance with the *Centers for Diseases Control and Prevention*⁶. Waist circumference was measured at the midpoint between the last rib and the iliac crest using an anthropometric measurement tape.

Oxygen Consumption

Oxygen consumption (VO_2), ventilation (VE) and respiratory oxygen equivalent (VE/VO_2) were measured using the VO2000 gas analyzer (Medial Graphics) during the incremental test on the treadmill (IMBRAMED, Master Super ATL). The Modified Balke protocol was used for the incremental test. This protocol involves a fixed speed of 3 mph, and 2.5% increases in incline every 2 minutes until maximum effort¹³. The test was discontinued when one or more of the following criteria were observed: voluntary fatigue or inability to maintain the pre-determined speed; or when one of the following criteria was demonstrated: exhaustion signs (pallor, abrupt reduction of exertion); VCO_2 / VO_2 ratio > 1.1 ; $> 90\%$ of estimated heart rate; chest pain report¹³.

Heart rate was measured using a heart rate monitor (Polar Electro Oy, Professorintie, FI-90440 Kempele, Finland). Peak oxygen consumption (VO_{2peak}) was determined as the highest VO_2 attained during the test¹³.

Blood analysis

Blood samples were collected from the antecubital vein in vacuum tubes, at two different moments. The first sample was collected 10 minutes before exercise and the second 20 minutes after exercise. Tumor necrosis factor alpha $TNF-\alpha$, and interleukins 6 (IL-6) and 10 (IL-10) were dosed by the chemiluminescence method using specific kits in the IMMULITE 1000 Immunoassay System equipment, SIEMENS.

Control Variables

Maturation was evaluated by the observation of secondary sexual characteristics according to the method proposed by Tanner¹⁴, in which adolescents self-report their development of pubic hair as one of five stages displayed in a set of pictures. Physical activity level was assessed using a pedometer (Yamax, SW700) for 7 (seven) consecutive days. The cutoff point adopted in this study for classifying the adolescents as insufficiently active was $<10,000$ steps/day as proposed by Tudor- Locke and Basset¹⁵.

Stress level was assessed prior to the beginning of each exercise session (visits 2 [two] and 3 [three]) using the stress scale for adolescents (ESA) developed and validated by Tricoli¹².

Statistical Analysis

Data were analyzed using the SPSS software (version 20.0). The results are presented as mean and standard deviation values. Two-factor analysis of variance (two-way ANOVA) for repeated measures was used to compare the values of inflammatory cytokines between different

exercise intensities (55% vs. 75%) and moments (pre vs. post). If Mauchly's sphericity test was violated, the Greenhouse-Geisser correction was adopted. When the F test identified an effect and/or interaction, the Bonferroni post hoc was applied to locate the differences between the means. Significance level was set at $p < 0.05$.

Results

Characteristics of the Participants

All the adolescents were classified as obese⁶ (BMI > 95th percentile), and 6 (six) were above the 99th percentile. Regarding waist circumference, 8 (eight) adolescents presented values above the 90th percentile¹⁶. In relation to physical activity and fitness levels, all participants were classified as insufficiently active^{15,13} and low cardiorespiratory fitness level. Of the total sample, seven adolescents were in stage four of sexual maturation and three in stage five (Table 1). Regarding the stress scale, adolescents presented similar stress levels (data not shown) in both physical exercise sessions.

Table 1. Characteristics of the obese adolescents

	Total (n=10)
Age (years)	15.90 ± 0.31
Height (m)	1.71 ± 0.10
Body Weight (kg)	104.93 ± 25.71
BMI (kg/m ²)	35.39 ± 5.42
WC (cm)	102.40 ± 11.47
VO _{2peak} (mL·kg ⁻¹ ·min ⁻¹)	27.46 ± 3.07
Steps/day	7088.87 ± 1843.34

Note: BMI (body mass index); WC (Waist circumference); Data are expressed as mean and standard deviation

Source: Authors

Post-exercise Inflammatory Markers

Acute aerobic exercise induced significant increases in IL-6 ($p = 0.005$; partial $\eta^2 = 0.362$; Table 2) between baseline and post-exercise for the moderate exercise session (55% of VO_{2peak}). However, for the vigorous exercise session (75% VO_{2peak}), no significant changes in inflammatory markers (TNF- α , IL-6 and IL -10) were observed between pre- and post-exercise (Table 2).

Table 2. Acute effects of different intensities of physical exercise on inflammatory markers

	55%	75%	ANOVA			
			Effect	F	p	(η^2)
TNF- α			Time	0.740	0.401	0.039
Pre	6.23 ± 1.64	6.82 ± 1.22	Intensity	0.001	0.972	0.000
Post	7.03 ± 1.49	6.48 ± 1.48	Time x Intensity	4.563	0.047	0.202
IL-6			Time	10.213	0.005	0.362
Pre	3.35 ± 1.53	3.33 ± 0.96	Intensity	0.040	0.843	0.002
Post	3.85 ± 1.73*	3.63 ± 1.09	Time x Intensity	0.638	0.435	0.034
IL-10			Time	0.031	0.863	0.002
Pre	5.00 ± 0.00	5.41 ± 1.29	Intensity	0.077	0.785	0.004
Post	5.36 ± 1.13	5.13 ± 0.41	Time x Intensity	1.969	0.178	0.002

Note: TNF- α (Tumor Necrosis Factor alpha); IL6 (Interleukin 6); IL10 (Interleukin 10). Data are expressed as mean and standard deviation, * intragroup difference in relation to pre-exercise ($p < 0.05$)

Source: Authors

Discussion

This study investigated the acute effects of continuous moderate- and high- intensity exercise on inflammatory markers in obese adolescents who were insufficiently active and presented low cardiorespiratory fitness.

In this study, 30 minutes of moderate intensity exercise resulted in significant changes in circulating levels of IL-6 ($p < 0.05$), and non-significant changes in TNF- α and IL-10. This response to moderate intensity exercise has also been reported in previous studies⁸.

Vigorous intensity exercise did not cause significant changes in cytokines measured in this study. The non-significant changes observed were a slight increase in IL-6 concentration (9%), and a decrease in TNF- α (5.2%) and IL-10 (5.4%), corroborating the findings of Ambarisha et al.¹⁷

Similar to the current study, Lau et al.¹⁸ also examined the acute effects of exercise on inflammatory markers in young people with chronic kidney disease. After 20 minutes of cycling at 50% of VO_{2peak} , a non-significant decrease was observed in TNF- α (4.5 to 4.2 picogram/milliliter [pg/ml]). With regard to vigorous intensity exercise, the study of Ulven *et al.*¹⁹ reported an increase in serum levels of TNF- α after 60 minutes of cycling at 70% of VO_{2max} . These disparities between studies may be explained in part by differences in the exercise protocols adopted.

Similar results to the present study in relation to TNF- α are presented in the literature²⁰, which shows that 30 minutes of walking at 75% VO_{2max} was not able to promote significant changes in the TNF- α marker. Comparing the effects of high-intensity intermittent exercise with continuous moderate intensity exercise on inflammatory and metabolic responses in young males, Cabral-Santos et al.⁴ demonstrated that in both sessions there was an increase in TNF- α immediately after the activity, and the high intensity activity resulted in a lower increase in TNF- α values compared to the moderate intensity activity⁴, reinforcing the findings of Nieman et al.¹¹, for whom acute modulations in TNF- α cytokine are related to the exercise intensity.

This study also found that serum IL-6 levels presented significant acute changes in response to moderate intensity physical exercise (55% VO_{2peak}). A single treadmill exercise session resulted in acute elevations of circulating IL-6, supporting the effects of physical exercise on the serum concentrations of this interleukin²¹.

Exercise-induced alterations in IL-6 between baseline and post-exercise were also demonstrated in studies with young adults^{20,22,23}, obese children²⁴, and in obese mice²⁵. In relation to the latter, Macpherson et al.²⁵ conducted a study on insulin action in adipose tissue and signaling of IL-6 after exercise in obese mice, the results indicated an improvement in the insulin signaling in adipose tissue as a result of an increase in the inflammatory markers IL-6 and IL-10. The increase in IL-6 circulatory concentrations in response to physical exercise has been shown to be beneficial to metabolic and immune mechanisms²⁶, as it leads to subsequent anti-inflammatory responses, as oppose to inflammation and insulin resistance that occur in the absence of physical exercise.

The IL-6 binds to proteins in the acute phase of inflammation caused by exercise. Increased concentrations of IL-6 induce the synthesis of the pro-inflammatory cytokines IL-1 and TNF- α antagonist receptors²⁶. Subsequent responses of the organism promote an anti-inflammatory environment. As for IL-10, previous evidence^{27,28} demonstrates that this cytokine is responsive to exercise, and that high intensity activity results in a greater increase of IL-10 compared to light and moderate intensity exercise. However, the exercise protocol used in the present study did not cause a significant increase in IL-10, supporting the hypothesis of Paulson et al.²⁹, in which the authors state that high-intensity exercise is needed ($\geq 85\%$ VO_{2max}) for significantly increasing the concentration of this cytokine.

Chronic low-grade inflammation is a physiopathological characteristic that is a determinant factor for the development of chronic diseases associated with obesity (diabetes mellitus type 2, NAFLD and cardiovascular disease)³⁰. Thus, monitoring of inflammatory biomarkers is essential for the prevention of cardiometabolic diseases and disorders, particularly those induced by obesity³⁰.

Regular physical exercise practice has a protective effect against the development of an array of chronic diseases, as it promotes a post-exercise anti-inflammatory state. In addition, exercise helps to reduce chronic low-grade inflammation by assisting with weight and fat mass reduction, generating a favorable metabolic adaptation in the organism in the long run³¹.

One limitation of this study was the lack of monitoring of cytokines levels at various post-exercise time points.

Conclusion

In conclusion, the results of this study indicate that a single exercise session (acute effect) was able to modulate inflammatory biomarkers in obese adolescents. This finding has important clinical implications, as it may help to control chronic low-grade inflammation related to obesity in adolescents.

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