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**Avaliação de Biomarcadores  
Cardiopulmonares, Metabólicos e  
Epigenéticos em Indivíduos com  
Esquizofrenia Submetidos a um  
Programa de Treinamento Combinado**

**UFCSPA**  
Universidade Federal de Ciências da Saúde  
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2021

**Avaliação de Biomarcadores Cardiopulmonares, Metabólicos e Epigenéticos em Indivíduos com Esquizofrenia Submetidos a um Programa de Treinamento Combinado**

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## RESUMO

A esquizofrenia se caracteriza como uma doença mental grave que leva o indivíduo ao isolamento social, ao sedentarismo e às várias comorbidades associadas como a obesidade e as doenças cardiovasculares. Esta tese teve como objetivo avaliar o impacto do treinamento combinado sobre a modulação de parâmetros epigenéticos, marcadores inflamatórios e de dano oxidativo, capacidade funcional e força muscular periférica em pacientes esquizofrênicos. Trata-se de um estudo quantitativo, intervencionista, descritivo e quase-experimental com pré e pós intervenção. Ao todo, 22 pacientes foram submetidos a um programa de treinamento combinado (exercícios aeróbicos e de força) durante 60 min, três vezes por semana e amostras de sangue, dados antropométricos, teste de caminhada dos 6 min e teste de força de preensão palmar foram coletados nos momentos pré-intervenção e 30, 90, 180, 270 dias após o início da intervenção. O treinamento combinado reduziu a massa corporal e o IMC em 90, 180 e 270 dias, aumentou a distância percorrida em 90, 180 e 270 dias e a força muscular periférica nos 30 dias após a intervenção. A atividade de CK aumentou em 90 dias, e a menor atividade de CK-Mb foi encontrada em 180 dias e 270 dias. Uma diminuição na atividade de HDAC2 foi encontrada em 180 dias. Além disso, foram encontrados um aumento significativo nos níveis de IL-10 em 90, 180 e 270 dias, uma redução significativa do TNF-alfa em 180, 270 e da leptina em 90 e 270 dias. Foram encontradas reduções significativas de TBARS em 180 e 270 dias e nitritos aumentados em 270 dias. Não ocorreram diferenças significativas nas IL-6, IL-33, no AOPP e no cortisol. Dessa forma, acredita-se que o treinamento combinado é uma estratégia de intervenção não farmacológica com a capacidade de modular diferentes fatores relacionados com a esquizofrenia contribuindo para a melhora do quadro geral de saúde, bem como, na redução de comorbidades associadas.

Palavras-chave: transtornos mentais; atividade física; fosfocreatina quinase; capacidade funcional; doenças psiquiátricas; epigenética.

## ABSTRACT

Schizophrenia is characterized as a serious mental illness that leads the individual to social isolation, physical inactivity and several associated comorbidities such as obesity and cardiovascular diseases. This thesis aimed to evaluate the impact of combined training on the modulation of epigenetic parameters, inflammatory markers and oxidative damage, functional capacity and peripheral muscle strength, in schizophrenic patients. It is a quantitative, interventional, descriptive and quasi-experimental study with pre and post intervention. At all, 22 patients underwent a combined training program (aerobic and strength exercises) for 60 minutes, three times a week. Blood samples and anthropometric data were also collected, in addition to the 6-minute walk test and handgrip strength test. All data were collected at the pre-intervention moments, 30, 90, 180 and 270 days after the beginning of the intervention. Combined training reduced body mass and BMI at 90, 180 and 270 days, increasing the distance covered at 90, 180 and 270 days and peripheral muscle strength at 30 after the intervention. CK activity increased in 90 days, and the lowest CK-Mb activity was found in 180 days and 270 days. A decrease in HDAC2 activity was found at 180 days. In addition, a significant increase in IL-10 levels was found at 90, 180 and 270 days and a significant reduction in TNF-alpha at 180, 270 and leptin at 90 and 270 days. Significant TBARS reductions were found at 180 and 270 days and an increase in nitrite at 270 days. There were no significant differences in IL-6, IL-33 and AOPP and cortisol. Thus, it is believed that combined training is a non-pharmacological intervention strategy with the ability to modulate different factors related to schizophrenia, contributing to the improvement of the general health condition as well as the reduction of associated comorbidities.

Keywords: mental disorders; physical activity; phosphocreatine kinase; functional capacity; psychiatric illnesses; epigenetics

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ACTH	Hormônio adrenocorticotrófico
AGAFAPÉ	Associação Gaúcha de Familiares de Pacientes Esquizofrênicos de Porto Alegre
AOPP	Oxidação protéica
BDNF	Fator neurotrófico do encéfalo
CAPES	Coordenação de Aperfeiçoamento de Pessoal de Nível Superior
CAT	Catalase
CID	Classificação Internacional de Doenças e Problemas Relacionados à Saúde
CNPQ	Conselho Nacional de Desenvolvimento Científico e Tecnológico
SNC	Sistema nervoso central
SZ	Esquizofrenia
DNA	Ácido desoxirribonucleico
DNMT	DNA <i>methyltransferase</i>
DSM	Manual Diagnóstico e Estatístico de Transtornos Mentais
EDTA	Ácido etilenodiamino tetra-acético
ELISA	Ensaio de imunoabsorção enzimática
FAPERGS	Fundação de Amparo à Pesquisa do Rio Grande do Sul
GSH-Px	Glutadiona peroxidase
HAT	Histona acetiltransferase
HDAC	Histona deacetilase
HPA	Hipotálamo-pituitária-adrenal
IL	Interleucina
IMC	Índice de massa corporal
NO	Óxido nítrico
PBMC	Células mononucleares do sangue periférico
SOD	Superóxido dismutase
TBARS	Substâncias reativas ao ácido tiobarbitúrico
TBA	Ácido tiobarbetúrico
TCLE	Termo de Consentimento Livre e Esclarecido
Th	Célula T auxiliar
TNF	Fator de necrose tumoral

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## 1 CONTEXTUALIZAÇÃO

Tem sido descrito que o equilíbrio entre mente e corpo é fundamental para uma melhor qualidade de vida. Estes campos, da saúde e do cognitivo, estão estreitamente vinculados, pois a mente compactua com o corpo e o corpo com a mente (MCCUTCHEON; REIS; HOWE, 2019). Naturalmente, não há separação entre corpo e psiquismo ou entre organismo e pensamento, uma vez que a vida é um processo de constantes trocas com o meio ambiente (RODRIGUES, 2006). Isso é particularmente importante em um mundo cada vez mais veloz, no qual o domínio da tecnologia e a necessidade de competências diversas tornam o indivíduo mais vulnerável ao estresse (GREENBERG, 2002).

Nesse mundo tão diverso se encontram inseridas pessoas com diversas doenças crônicas que envolvem o desequilíbrio entre mente e corpo onde destacam-se a esquizofrenia, o autismo, o transtorno bipolar severo e a depressão. Além desses desequilíbrios, os indivíduos apresentam maiores chances de desenvolverem outros problemas de saúde, além da doença mental, o que influencia diretamente em sua qualidade de vida (SUN; KENNEDY; NESTLER, 2013).

Múltiplas causas contribuem para a doença mental. Achados recentes apontam o desequilíbrio de mecanismos epigenéticos na fisiopatologia de doenças mentais graves como esquizofrenia, depressão e transtorno bipolar (SUN; KENNEDY; NESTLER, 2013; NESTLER et al., 2002). Dentre essas modificações, destacam-se a acetilação de histonas, marcador associado ao aumento do processo transcricional; e a metilação do ácido desoxirribonucleico (DNA), associado ao silenciamento gênico (BIRD, 2007). Nesse contexto, a redução na expressão do gene do fator neurotrófico do encéfalo (BDNF) tem sido sugerida como um potencial fator envolvido na fisiopatologia de diversos transtornos mentais graves (RAY et al., 2011; CARLINO et al., 2011).

Ainda relacionado com a modulação gênica, devemos considerar o papel dos glicocorticóides na regulação da expressão de genes relacionados com a resposta imune, mais especificamente aos processos inflamatórios, uma vez que a desregulação de receptores de glicocorticóides tem sido observada em pacientes com esquizofrenia e bipolaridade (SINCLAIR et al, 2011; RIVES et al., 2009). Os receptores do cortisol modulam a expressão de vários genes pró-inflamatórios, entre

eles as interleucinas (IL): IL-1B, IL-6 e IL-8. A modulação desses marcadores, quando observada em indivíduos saudáveis, contribui para a redução de processos inflamatórios, impedindo a liberação exacerbada destas substâncias. Por outro lado, a perda desse controle leva à liberação excessiva destas IL, o que também tem sido relatado como contribuidor para o desenvolvimento de doenças mentais graves (MUKAIDA et al., 1994; VERHOOG et al., 2011). Dessa maneira, sugere-se que os marcadores epigenéticos e inflamatórios interagem entre si, participando da fisiopatologia de doenças mentais graves e de longa evolução. Contudo, os dados ainda não são conclusivos, e, portanto, merecem ser explorados.

Além dos problemas moleculares, celulares e fisiológicos, cabe descrever que as dificuldades sociais são notadamente preocupantes nesses indivíduos, o que pode ser exemplificado pela árdua inserção no mercado de trabalho e pelas dificuldades no relacionamento inter-pessoal. Para Rodrigues (2006), esses indivíduos tendem a não interagir com a comunidade, o que resulta no isolamento social, podendo justificar o fato de que grande parte deles não praticam qualquer atividade física regular (RODRIGUES, 2006). Esse dado é relevante, uma vez que o sedentarismo traz inúmeros prejuízos à saúde contribuindo para as comorbidades associadas às doenças mentais. Especificamente em sujeitos com transtornos mentais, a inatividade física resulta em aptidão aeróbia reduzida, perda dos reflexos posturais, metabolismo lipídico alterado, balanço nitrogenado negativo, perda de massa muscular e redução de cálcio ósseo. Além disso, é fator de risco para diversas condições clínicas degenerativas e aterogênicas, como aterosclerose, osteoporose, fadiga crônica e obesidade (TEIXEIRA; ROCHA, 2013). Nahas (2001) cita, ainda, que na medida em que o indivíduo declina fisicamente pode haver uma deterioração concomitante na sensação do bem-estar, resultando em autoestima precária, ansiedade, fadiga, estresse, depressão e declínio da vitalidade.

Por outro lado, a prática de atividade física, além de contribuir para a manutenção da boa forma do corpo humano, é um meio eficaz para preservação do equilíbrio do corpo e da mente, o que pode prevenir ou estabilizar várias doenças crônicas, tanto físicas quanto psicossociais (PLOWMAN; SMITH, 2009). Assim, fica evidenciada a importância da prática e do incentivo a essa população no que concerne à realização de atividade física, contribuindo para a ampliação de seus espaços de

socialização e para a modulação de variáveis fisiológicas, e assim, melhorando sua qualidade de vida.

Logo, os promissores resultados pré-clínicos citados acima servem como justificativa para a realização de novos estudos relacionados aos efeitos do exercício físico sobre a modulação de parâmetros epigenéticos, agora conduzidos com humanos. Dentre as populações a serem alvo dessas pesquisas, destacam-se os indivíduos com transtornos mentais graves e de longa evolução, como a esquizofrenia, cuja fisiopatologia tem sido associada às alterações em mediadores inflamatórios, epigenéticos e de estresse oxidativo, assim como, no perfil celular imunológico e bioquímico.

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## 2 OBJETIVOS

### 2.1 OBJETIVO GERAL

Avaliar o impacto do treinamento combinado sobre a modulação de parâmetros epigenéticos, marcadores inflamatórios e de dano oxidativo, capacidade funcional e força muscular periférica, em pacientes com transtornos mentais graves e de longa evolução.

### 2.2 OBJETIVOS ESPECÍFICOS

- Verificar os dados antropométricos antes do início do programa 1, 3, 6 e 9 após um programa de treinamento combinado em indivíduos esquizofrênicos.
- Avaliar a capacidade funcional dos pacientes, 1, 3, 6 e 9 meses após um programa de treinamento combinado em indivíduos esquizofrênicos.
- Avaliação da força muscular periférica, 1, 3, 6 e 9 meses após um programa de treinamento combinado em indivíduos esquizofrênicos.
- Avaliar os níveis de citocinas anti e pró-inflamatórias antes do início do programa, 1, 3, 6, e 9 meses após um programa de treinamento combinado em indivíduos esquizofrênicos.
- Analisar a modulação de marcadores de dano oxidativo antes do início do programa, 1, 3, 6, e 9 meses após um programa de treinamento combinado em indivíduos esquizofrênicos.
- Quantificar a atividade da HDAC antes do início do programa, 1 e 6 meses após um programa de treinamento combinado em leucócitos de indivíduos esquizofrênicos.
- Quantificar os níveis de cortisol antes do início do programa, 1 e 6 meses após um programa de treinamento combinado em leucócitos de indivíduos esquizofrênicos.

### 3 ARTIGO 1

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#### **Combined exercise protocol improves the functional capacity and systemic damage biomarkers in schizophrenic patients: A clinical longitudinal study.**

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## **Abstract**

**Background:** Schizophrenia is a mental illness characterized by severe psychosocial changes. Schizophrenic individuals have reduction in social interaction, which leads them to sedentary lifestyle. Physical exercise also results in improved functional capacity, cognition and body composition in this population; however, the ideal exposure time and type of training are not yet defined for people with mental illness.

**Objective:** This study aimed to evaluate the effect of a 9-month combined exercise protocol on peripheral muscle strength, functional capacity, body composition and systemic damage biomarkers in schizophrenic individuals. **Methods:** This is a

quantitative, interventionist, descriptive and quasiexperimental study. Participants were submitted a physical training program with 60 min duration, three times a week.

6 min walking test, handgrip strength test and analysis of creatine kinase (CK) and creatine kinase Mb (CKmb) were performed pre-intervention 30, 90, 180 and 270 days

after the beginning of the intervention. **Results:** Combined training reduced body mass and BMI at 90, 180 and 270 days compared to baseline. There was an increase in the distance covered at 90 days, 180 days and 270 days when compared to the pre-intervention period. The improvement in peripheral muscle strength was observed only

30 days after intervention. The CK activity increased at 90 days of combined training compared to before intervention. Lower CK-Mb activity was found at 180 days and 270

days. **Conclusion:** The applied training protocol was able to reduce cardiovascular risk, increase functional capacity and physical fitness of schizophrenic individuals. 90 days is necessary to modulate their body mass.

**Keywords:** Schizophrenia, Exercise, functional capacity, comorbidity, mental disease, creatine kinase, Combined exercise

## 1. Introduction

Schizophrenia (SZ) is a serious long-term mental illness that, according to the World Health Organization, affects 1% of the population. It is characterized by severe psychosocial changes in thinking, brain functioning, perception and behavior [1]. As a result, schizophrenic individuals have difficulties in concentration, understanding and also a reduction in social interaction, which leads them to isolation and sedentary lifestyle [2] and its etiology is related to biological and environmental factors. Long-term care and disease management are critical, as debilitating treatment side effects often affect the patient's physical and functional capacity [3]. In fact, current evidence indicates impaired muscle force-generating capacity and functional performance in patients with SZ [4]. Unhealthy living habits, such as low level of physical activity, contribute to the development of harmful health conditions [5].

In this context, it is important to note that physical inactivity is more prevalent in subjects with mental disorders when compared to the general [6]. A high prevalence of smoking, poor nutrition, impaired lipid profile and obesity are aspects observed in SZ [7], contributing to the increasing the risk of developing associated comorbidities [8]. In this sentence, individuals with SZ have a higher prevalence of metabolic syndrome [9], type II diabetes mellitus [10] and cardiovascular disease [11], in addition to weight gain that is applied to 72% of patients taking antipsychotic medication [12] which is also one factor related to weight gain and metabolic disorders.

High systemic levels of inflammatory cytokines seem to be associated with SZ and potentiated comorbidities, especially

the accumulation of adipose tissue [13,14]. Patients who demonstrate high systemic levels of Creatine Kinase (CK) during acute psychosis also demonstrate persistently moderate levels of CK during non-psychotic periods [15], with a direct

correlation between increased symptoms and increased CK [16]. On the other hand, it is known that exercise is able to modulate inflammatory parameters and the physiological adaptations observed attenuate comorbidities.

Furthermore, several studies have been highlighting that physical activity in this population is related to improved mental health, cardio-metabolic and respiratory capacity [17], with clinical improvements being observed when performing at least 90 minutes of moderate to vigorous physical exercise per week [18]. Physical exercise also results in improved functional capacity, cognition and body composition in SZ individuals [19, 20]. It was previously reported that a combined exercise protocol (CEP), specifically the combination of 40 minutes of aerobic exercise followed by 20 minutes of resistance exercise, applied for six months, resulted in improved cardiorespiratory fitness in this population [21]. It was also observed that this regimen is able to ameliorate strength levels, improve schizophrenia symptoms and modification of body composition when performed for at least 12 weeks [22, 23].

Despite these data, evidence demonstrating the short, medium and long term outcomes of a combined exercise protocols on functional capacity, body composition and inflammatory biomarkers in this population are scarce. Then, this study aimed to analyze the time-course effect of a combined exercise protocol on the functional capacity, handgrip strength and muscles damage in individuals with SZ.

## **2. Materials and Methods**

### **2.1 Participants and Ethical Considerations**

This is a quantitative, interventionist, descriptive and quasi-experimental study. The study included 22 individuals diagnosed with schizophrenia, of both genders, aged between 30 and 60 years with severe and long-term mental disorders according to the International Classification of Diseases (ICD-10) with medical diagnosis. They should be sedentary for a minimum of six months, do not realizing another physical exercise modality during the trial participation, having regular medical treatment and medication that certifies that the individual is able to participate of the proposed physical activity program. Exclusion criteria were individuals who reported diseases that prevented the practice of physical exercise and / or have a medical contraindication, with a history of autoimmune diseases, cardiovascular problems, presence of joint, musculoskeletal or neoplastic diseases that prevented them from perform the exercises and individuals who did not agree to participate in the proposed physical activity program. We also used as exclusion criteria the frequency below 90% of the training protocol.

The study was approved by the Research Ethics Committee at the Federal University of Health Sciences of Porto Alegre, under number 2,464,092, and all procedures followed National Health Council Resolution 196/96, which regulates the research in human beings in the country. Those responsible for the participants signed the agreement to participate in the study.

## 2.2 Experimental draw

The effect of combined exercise protocol on peripheral muscle strength, body composition and functional capacity was evaluated at different time-points: pre-intervention, 30, 90, 180 and 270 days after the beginning of the intervention. For CK-MK analysis, blood samples were also collected in these moments. The CEP were carried out in group, 3 times a week, lasting one hour . The physical activity program

as well as the evaluations were carried out at the facilities of the Gaúcha Association of Family Members of Schizophrenic Patients (AGAFAPE).

### 2.3 Physical Activity Protocol

For this study, we used an adapted protocol previously proposed by Lavratti [23]. Due to the psychological condition of the population studied, there was no differentiation of burden between participants. In order to prevent the patients from feeling underestimated and or persecuted by the professionals who applied the protocol, all individuals were subjected to identical intensity, making it impossible the load progress and individualization of the training.

The protocol consisted in one week of adaptation and subsequently 36 weeks of training with three weekly sessions of during 60 minutes in group. The training consists in sessions started with 30 minutes of aerobic exercise, where circuits with obstacles were realized, including jump over broom handles, pass over a rope allocated 30 cm from the ground, slalom on juxtaposed bottles of water, parked race, polichinels, jumps and dance. It's important to remind the necessity of the creativity in the variation of the proposed activities in different sections, once the participants presented rejection to certain exercises that were being repeated over the weeks and the inclusion of playful elements increased adherence on the proposed exercises. Therefore, the individuals were submitted to 30 minutes of resistance training with 3 sets time-controlled with 7 exercises involving large and small muscle groups such as: push-up adapted with the hand on the wall and body tilted (pectoralis major, pectoralis minor, triceps brachii), shoulder abduction (deltoid, trapezius), squat ( femoral quadriceps, gluteus maximus, sural triceps), knee flexion (ischia-tibial), elbow flexion (biceps brachii), elbow extension over the head (triceps brachii) and plantar flexion

(sural triceps). In the end of the session, they had final stretching and returned to calm. The intervals between resistance exercises were approximately 30 seconds.

The exercises series were monitored per time and not by the number of repetitions were: More accessibility for the group activity occurred in a coordinated way; some patients presented serious dyslexia that made it impossible for them to count the repetitions number they realized when doing the exercises. In the adaptive period, free active exercises were performed without resistance. Afterwards 1kg dumbbells made with water bottles were added to the abduction exercises of shoulder, elbow flexion and elbow extension over the head. The series time gradually increased as patients reported a low level of demand, starting in 30 seconds and progressing over time to 40, 50 to 1 minute. The utilization of music during the exercise execution showed up to be one interesting strategy to avoid distractions, once the patients had understood that while the music were playing they should realize the exercise and, as soon as it stopped, they were able to rest. Exercise interventions were supervised by a responsible physical therapist, that was always the same since the beginning of the study. Blood pressure, BORG effort perception and peripheral oxygen saturation were monitored before and after the interventions, and heart rate was monitored throughout the exercise.

#### 2.4 Biomarker Measurements

The blood was collected at the antecubital region by a properly qualified professional, as well as the location, which was prepared, sanitized and refrigerated for the procedure realization. The time estimated for it was 15 minutes. In all collections, 15 ml of blood was taken using venous canulation with disposables needles and syringes.

For CK-MB and sCK analyzes, spectrophotometer method was used in an automatized equipment called Bioclin BS-120, according to the supplier recommendations. The CK were dosed in the participants serum with the use of one Automatic Biochemical Analyzer from BioSys/Kovalent, model Miura One with CK-NAC DGKC/IFCC kit, code 2020075F from Kovalent.

## 2.5 Functional Outcomes

Peripheral muscle strength was assessed using the handgrip strength (HGS), which was measured using the Jamar® Hand Dynamometer (Sammons Preston Jamar Hand Dynamometer), following the recommendations of the American Society of Hand Therapists (ASHT). The individual had to be seated in a chair, with the shoulders positioned in a neutral position, one hand resting on the thigh while the evaluated limb elbow was maintained at 90 ° with neutral forearm rotation and up to 30 ° of wrist extension to perform an isometric type muscle contraction force. Three measurements were performed, with recovery time between one minute measurements and the best of the three attempts was used as a reference measurement [24]. It was done in pre-intervention, 30, 90, 180 and 270 days after the beginning of the intervention.

For functional capacity evaluation, it was used The 6-minute walk test, which was performed following the recommendations established by the American Thoracic Society [25]. Prior to the beginning of the test, patients were instructed on the purpose and implementation of the test and advised to slow down or stop if they had symptoms such as chest pain, breathing disorder or severe muscle pain. However, the timer did not stop at any of these events. All subjects received verbal stimuli to help them

complete the test. Heart rate, pulse oximetry SpO<sub>2</sub> (model 1001 Morryalpiranga, São Paulo, Brazil) and exertion perception were measured by the modified Borg scale (data not shown). After 6 minutes, the evaluator recorded the distance traveled by the volunteer.

To measure height, a wall-mounted tape measure was used. The patient was positioned upright with legs and feet parallel, weight distributed on both feet and arms extended at the side of the body, head at right angles to the neck and looking at a fixed point at eye level. Weight was measured using a Gama Fit Ultra portable electronic scale and body mass index (BMI) calculated by the equation weight in kg divided by height in square meters. Participants were instructed not to change the diet and eating habits that they had pre-intervention. Both were taken in pre-intervention, 30, 90, 180 and 270 days after the beginning of the intervention.

## 2.6 Data analysis

The statistical program SPSS 20.0 (SPSS Inc., USA) was used for the analyzes. Data normality was verified by the Shapiro-Wilk test, and data were presented as mean  $\pm$  standard deviation. An analysis of variance (ANOVA) of repeated measures followed by a Bonferroni post test for multiple comparisons was adopted for comparisons over the intervention time. A significance level of  $p \leq 0.05$  was adopted. The effect size (ES) was used to determine the magnitude of changes between evaluations of the protocols. Threshold values were  $< 0.35$  (trivial), 0.35–0.8 (small), 0.8–15.0 (moderate) and  $> 15$  (large). G Power (version 3.1.3; Franz Faul, Kiel, Germany) was used to calculate the sample size of the study and indicated that a minimum of 18 participants would be enough to detect differences in 6MWT (a primary outcome variable) [26] with a power of 95%, 5% significance level, and an effect size of 0.91.

### 3. Results

A total of 22 patients participated in the present study, with a mean age of  $45.57 \pm 8.88$ , 31.8% female and 68.2% male. The protocol had high adherence by all participants since only 1 had to leave the study due to the diagnosis of renal neoplasia. Table 1 shows the sample characteristics of the patients.

As shown in table 1, there was a statistically significant reduction in BMI and body mass 90 (Body mass,  $p=0.04$ ;  $ES=0.24$ ; BMI,  $p=0.04$ ;  $ES=0.23$ ), 180 (Body mass,  $p=0.03$ ;  $ES=0.3$ ; BMI,  $p=0.04$ ;  $ES=0.28$ ) and 270 (Body mass,  $p=0.04$ ;  $ES=0.31$ ; BMI,  $p=0.04$ ;  $ES=0.29$ ) days after the intervention compared to the baseline period.

Figure 1 illustrates the effect of intervention on functional capacity. The distance traveled increased at 90 days ( $430.2 \pm 112.3$  m;  $p = 0.001$ ;  $ES=0.71$ ), 180 days ( $511.1 \pm 130.6$ m;  $p = 0.001$ ;  $ES=1.13$ ) and 270 days ( $492.9 \pm 119.6$ m;  $p = 0.001$ ;  $ES=1.26$ ) compared with the pre-intervention period ( $356.6$ m,  $\pm 89.25$ ). A significant increase in peripheral muscle strength was observed only 30 days after intervention ( $25.62 \pm 11.71$  J to  $27.88 \pm 11.58$  J;  $p = 0.04$ ) (table 1).

Figure 2 highlights the systemic activities of CK (Fig.2A) and CK-Mb (Fig. 2B) in response to combined exercise protocol. The CK activity increased at 90 days compared to before intervention ( $p=0.008$ ;  $ES=0.35$ ). Conversely, lower CK-Mb activity was found at 180 days ( $p=0.04$ ;  $ES= 0.39$ ) and 270 days ( $p=0.04$ ;  $ES= 0.40$ ) after training.

### 4. Discussion

The main objective of the study was to evaluate in the short, medium and long term effects of a combined exercise protocol on the functional capacity, anthropometric parameters and the muscular damage profile of individuals with schizophrenia. In summary, the intervention induced a significant reduction in the body composition

levels of schizophrenic individuals when applied for more than 90 days. After 90 days it was observed a significant increase in the distance traveled by patients in the 6MWT, that remained until the last assessment, performed 270 days after the beginning of the intervention, with a progressive increase in effect size. The peripheral muscular strength raised in the first moment that the individuals were exposed to the exercise. CK showed a significant increase with a peak in 90 days, being reduced after this moment. CK MB had significant reduction in 180 and 270 days.

Our anthropometric results corroborate with the findings obtained by Battaglia et al. [19], where schizophrenic individuals who underwent soccer training for 3 months at a frequency of twice a week, with interventions of 100 to 120 min, presented a significant reduction in BMI. Also, Beebe et al. [27] described the effects of a treadmill walking program with frequency of 3 times per week with 30 minutes of aerobic activity per session for 4 months in outpatients with schizophrenia. The authors observed a significant reduction in fat percentage, but without changes in BMI. These findings suggest that regardless of the exercise protocol used, aerobic exercise interventions lasting longer than 90 days are effective in inducing improvement of anthropometric data. Based on these findings, we believe that aerobic activity time is related to weight loss, considering that Marzolini et al. [26] did not observe significant body weight reduction after performing a CEP protocol for 90 days, with 2-week interventions.

Regarding to the improvement of functional capacity, our findings are in agreement with the data obtained by Marzolini [26] who observed improvement in cardiorespiratory conditioning. In a study with schizophrenic individuals performing aerobic exercise twice a week for 6 months, found results equivalent to those of our study regarding cardiorespiratory fitness.

Handgrip strength (HGS) is a way of quantifying peripheral muscle strength, which can be interpreted as autonomy in daily living activities as well as cardiovascular protection. This marker, as concluded in Bohannon [28] systematic review, can be considered a useful vital sign for screening middle-aged and elderly adults, including predictive validity. Its reduction is linked to a range of health outcomes, including higher morbidity and mortality rates [29]. Gale et al. [30] evaluated 800 individuals over 65 years of both sexes in order to investigate the relationship between HGS, body composition and specific cause of mortality. Values lower than 26kg for men and 16kg for women were found to be associated with increased all-cause mortality and cardiovascular disease. Our results show that the intervention resulted in an increase in HGS, which started with an average of 25.5kg and after 30 days the average was significantly higher (27.7). Even with the reduction, the values remained above 26. We believe that the adaptation to exercise and the non-progression load is related to the reduction and stabilization of the force values in the other times evaluated. To date, there are no evidence in the literature that evaluate hand grip strength in individuals with long-term severe mental disorders, and this study is a pioneer in the evaluation of this parameter of functional capacity in this population.

In sedentary women, the combined treatment, individualized with charge progression, had taken a higher development of the cardiorespiratory capacity, in the first four weeks achieved 6,9%, getting to 8,5% improve in 60 days [31]. There was also an important progress in the muscle strength. Our results suggest one delayed cardiorespiratory adaptation in the schizophrenic individuals. Despite the low charge used, there is a strength improvement with the resistance work in a short period of the exercise, does not showing any significant loss over the months. We believe that the charge progression is essential for the positive results in a medium and long deadline

According to Clarkson et al. [32], who describe three profiles related to the sCK peak, the present study sample presented mean basal levels, in agreement with previous studies that indicate increased levels in schizophrenic patients (39). There are no studies that measure the modulation of these markers in response to physical exercise protocols, indicating the pioneer data of the current research.

It is known a positive relationship between load and volume of resistance exercises and serum CK [34, 35], individuals with low levels of physical activity are more susceptible to muscle damage [32], however, contrary to what is observed in healthy individuals, whose initial increase in creatine occurs in the first 6 weeks [16] the peak of Muscle injury showed a delayed response. The lower mechanical load applied may be related to this variation in the serum CK peak time, considering that the increase in CK after exercise is highly variable and affected by individual exercise factors [33]. Finally, the reduction in cardiac damage markers and the increase in the distance covered in the 6MWT observed in our study suggest an adaptive cardioprotective response after 90 days of combined exercise [35].

Finally, a remarkable point to discuss is the high patient's attendance to the intervention. Our findings are in agreement with other studies based on structured, supervised and group based exercise programs [36], [37]. Then, we might propose that interventions with this profile could result in best treatment adherence compared to those characterized as non-structured and non-group-based [38].

## **5. Conclusions**

Summarizing, our results demonstrated that a combined exercise protocol induced time-dependent improvements in anthropometric marks, functional capacity

and physical fitness of schizophrenic individuals, contributing to reducing cardiovascular risk.

## 6. Study limitations

Impossibility of progression and individualization of the load used in resistance exercises. Due to the psychological and cognitive aspects of the studied population, it was not possible to perform more complex exercises and activities, and these factors did not allow us to make a detailed assessment of body mass.

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**Tables**

Table 1. Characterization of the studied individuals

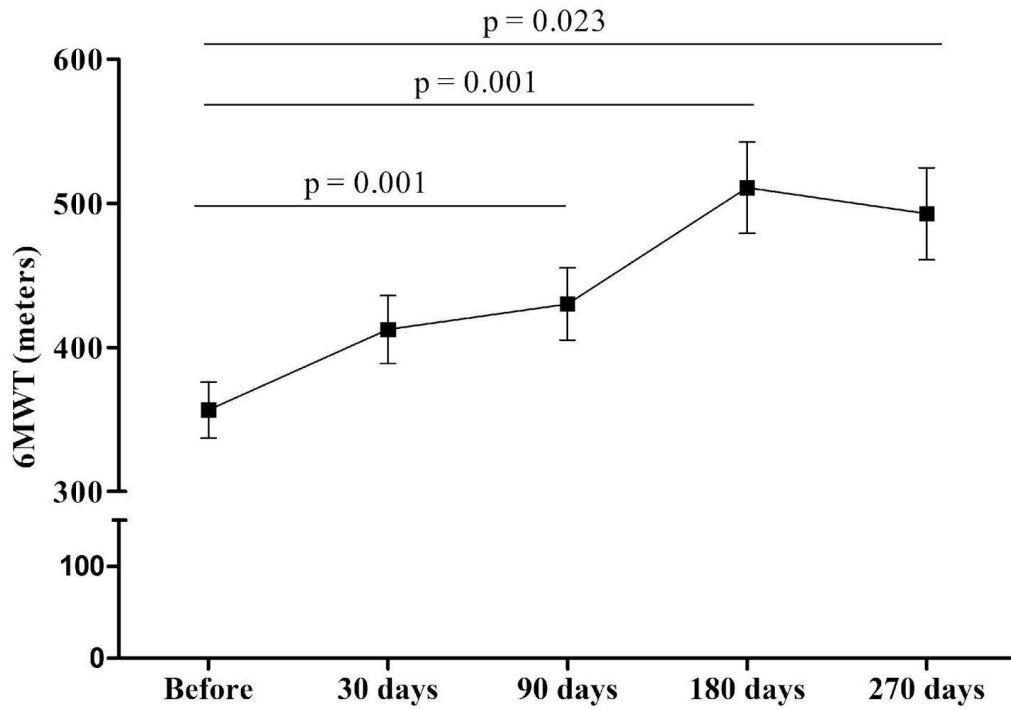
	Pre	30 days	90 days	180 days	270 days
Gender (male/female)	15/7	-	-	-	-
Age (years)	45,54 ± 8,88	-	-	-	-
Time of diagnosis (years)	27,45 ± 11,50	-	-	-	-
Height (m)	1,69 ± 0,07	-	-	-	-
Body mass (Kg)	66,23 ± 9,98	65.09 ± 10,20	63.75 ± 10,19 *	63,18 ± 10,21*	63.04 ± 10,06*
BMI (kg/m <sup>2</sup> )	24,00 ± 3,80	23,58 ± 3,87	23,09 ± 3,87*	22,89 ± 3,90*	22,84 ± 3,87*
Handgrip strength (Kg)	25.62 ± 11.71	27.88 ± 11,58 *	27.02 ± 9.67	26.91 ±10,11	26,98 ± 9,97

Data presented as mean ± standard deviation

\* It shows a significant difference compared to the pre-training moment (p< 0.05)

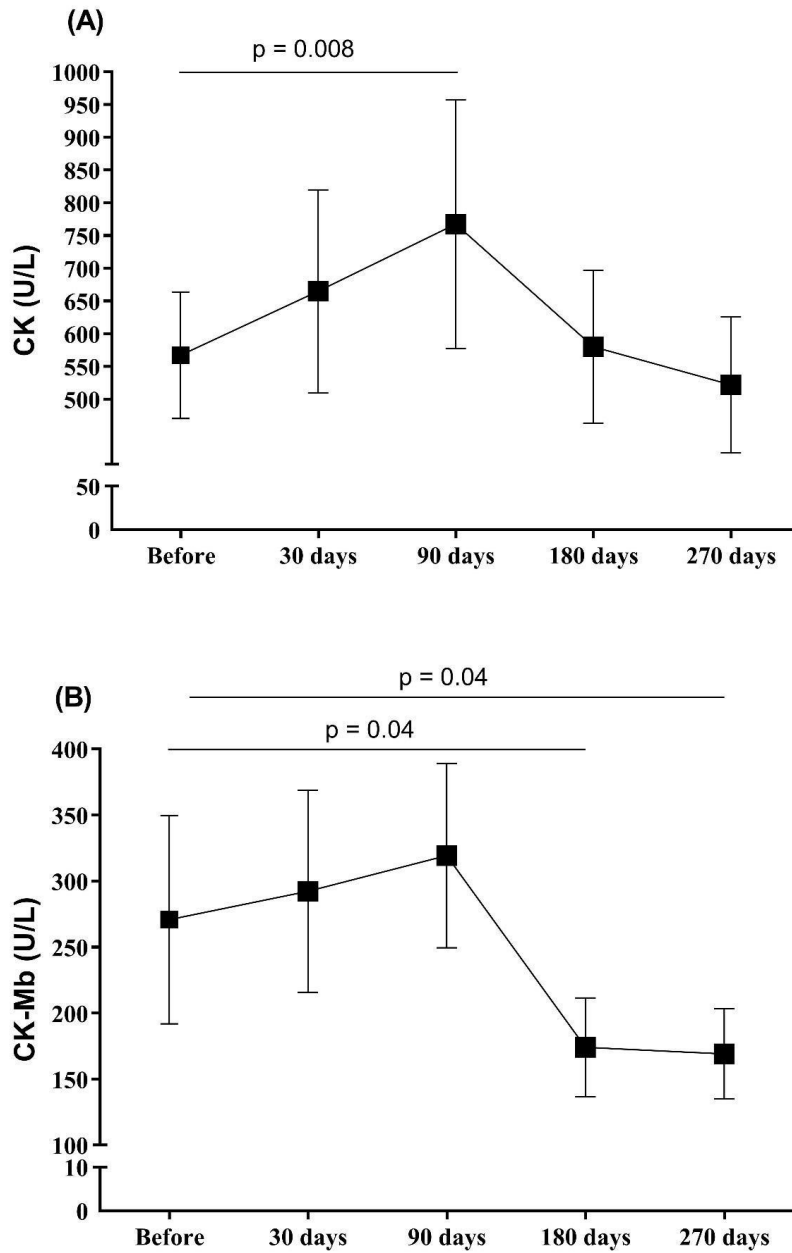
**Figures**

Figure 1. Effects of concurrent exercise protocol on functional capacity in schizophrenic individuals.



Statistical differences are indicated at time 90, 180 and 270 days when compared with the pre-intervention period.

Figure 2. The progress of CK and CK-Mb biomarkers in schizophrenic individuals.



Statistical differences are indicated at time (Figure 2A) 90 days and (Figure 2B) at times 90 and 270 days when compared with the pre-intervention period.

## 4 ARTIGO 2

Artigo a ser submetido ao periódico Journal of Nervous and Mental Disease. Fator de Impacto: 2.254. Qualis: A2.

### COMBINED TRAINING MODULATES SYSTEMIC LEVELS OF CYTOKINES IN PATIENTS WITH SCHIZOPHRENIA.

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#### Abstract

The aim of this study was to investigate the effect of combined training on cytokines and oxidative stress markers in schizophrenic patients. It is a quantitative, interventional, descriptive and quasi-experimental study with pre and post intervention. 14 individuals (age  $43.12 \pm 6.25$ ; 75% male) were evaluated through blood analysis at the following times: pre-intervention, 30, 90, 180 and 270 days. A significant increase in IL-10 levels was found at 90 ( $p = 0.038$ ), 180 ( $p = 0.033$ ) and 270 ( $p = 0.027$ ) days. There was a significant reduction in TNF-alpha in 180 ( $p = 0.02$ ), 270 ( $p = 0.001$ ) and leptin in 90 ( $p = 0.02$ ) and 270 ( $p = 0.028$ ) days. Significant TBARS reductions were found at 180 ( $p = 0.034$ ) and 270 ( $p = 0.042$ ) days with an increasing in nitrite at 270 ( $p = 0.03$ ) days. There were no significant differences in IL-6, IL-33 and AOPP ( $p > 0.05$ ). It is suggested that the combined training promoted a beneficial effect depending on the intervention time, modulating the inflammatory and oxidative response.

Keywords: schizophrenia, cytokines, combined training, oxidative stress.

## INTRODUCTION

Schizophrenia is a serious psychiatric condition that affects approximately 24 million individuals in the world (American Psychiatric Association, 1994). It is characterized by symptoms such as hallucinations, delusions, cognitive impairment and mainly a limitation in social function that leads to the isolation of schizophrenic patients (Berberian et al, 2019). Antipsychotic treatment associated with a decrease in physical activity in these patients leads to an increase in the prevalence of obesity and other comorbidities, such as cardiovascular diseases and insulin resistance, thus providing a chronic inflammatory condition and changes in the redox balance (Tian et al, 2020). An inflammatory environment with intense oxidative conditions can be considered a factor in the progression of schizophrenia. This is due to an immune imbalance caused by an increase in the levels of pro-inflammatory cytokines in combination with a reduction in the concentrations of anti-inflammatory molecules that compromise brain neurotransmission (Rodrigues-Amorim et al, 2018). Studies report that patients with schizophrenia have increased concentrations of proinflammatory cytokines (Noto et al, 2015) and abnormal antioxidant defenses in peripheral blood (Maas et al, 2017). Evidence points out that oxidative damage plays an important role in the pathophysiology of schizophrenia (Obata, 2002). The same occurs when there is an imbalance between antioxidant and prooxidant processes, or due to the inefficiency of antioxidant defenses, which can lead to pathophysiological processes for self-oxidation of some neurotransmitters (Thompson et al, 2001).

According to the guidelines of the European Psychiatric Association, physical exercise can be a non-pharmacological strategy to reduce psychiatric symptoms and improve cognitive functions in different subdomains, such as memory and concentration (Stubbs et al, 2018). Physical exercise is capable of promoting immunoprotective and immunoregulatory effects through the balance between pro and anti-inflammatory cytokines resulting in a systemic reduction of inflammation (Thielen et al, 2016). In addition, it can offer neuroprotective effects to

individuals, reducing the damage caused by oxidative damage (Seetharamaiah et al, 2010).

The present study was conducted to investigate the effect of a chronic combined exercise program on the systemic levels of pro and anti-inflammatory cytokines and on oxidative stress markers in schizophrenic patients.

## **MATERIALS AND METHODS**

It is characterized by a quantitative, interventionist, descriptive and quasiexperimental study with pre and post intervention. The convenience sample was used, based on the definition of Fletcher and Fletcher (2006). Thus, the sample was of the voluntary non-probabilistic type. The study included 14 individuals diagnosed with Schizophrenia, of both genders, aged between 30 and 60 years. The study included subjects with severe and long-term mental disorders with medical diagnosis according to the International Classification of Diseases (ICD10). Individuals should be sedentary for a minimum of six months, with regular medical and medication monitoring and able to participate in the proposed physical activity program. Exclusion criteria were individuals with diseases that make physical exercise unfeasible and / or have a medical contraindication, history of autoimmune diseases, cardiovascular problems, presence of joint, musculoskeletal or neoplastic diseases that prevented them from performing the exercises and individuals who did not agree to participate in the proposed physical activity program.

The study was approved by the Research Ethics Committee of the Federal University of Health Sciences of Porto Alegre, under number 2,464,092, and all procedures followed Resolution 196/96 of the National Health Council, which regulates the research in human beings in the country. Those responsible for the patients were invited to sign the Informed Consent Form (ICF).

### **Experimental drawing**

The effect of exercise on inflammatory and oxidative parameters was assessed at different times: pre-intervention, 30, 90, 180 and 270 days after starting the intervention. The physical activity program, as well as the evaluations, were carried out at the Association of Family Members of Schizophrenic Patients (AGAFAPE)

### **Physical Activity Protocol**

For this study, an adapted protocol was used, based on the study by Lavratti et al. 2017, of combined physical exercise, where the combination of resistance exercise associated with aerobic exercise performed in group was used. Due to the psychological condition of the population studied, load differentiation among participants proved to be a problem. In order to prevent patients from feeling underestimated and / or persecuted by the professionals who applied the protocol, all patients were submitted to identical intensity and load, making it impossible to adequately progress and individualize the training.

The combined physical exercise protocol consisted of an adaptation week for individuals to become familiar with the proposed activities. The standardization of training was 36 weeks of training, with three weekly sessions of 60 minutes. The combined training consisted of 30 minutes of aerobic circuits that included walking, jumping and ladder training, carried out in a playful manner, with permanent verbal stimuli and recommendations to try to reach their physical limit, aiming to provide greater well-being and get their participation; followed by 30 minutes of resistance training with 3 series of controlled time (for reasons of greater accessibility for group activity, some participants had severe dyslexia), with 7 exercises involving large and small muscle groups such as: hand-adapted push-up on the wall and inclined body (pectoralis major, pectoralis minor, triceps brachii), abduction of the shoulder (deltoid, trapezius), squat (quadriceps femoris, gluteus maximus, triceps sural), knee flexion (ischio-tibial), flexion of the

elbow ( biceps brachii), elbow extension over the head (brachial triceps) and plantar flexion (sural triceps). Final stretching was performed at the end of each training. The intervals between resistance exercises were approximately 30 seconds. In the adaptive period, free active exercises were performed without adding resistance. Subsequently, 1 kg dumbbells made with water bottles were added to the shoulder abduction, elbow flexion and elbow extension over the head exercises. The time of each series gradually increased (30, 40.50 and 60 seconds) as the patient reported a low level of demand. The inclusion of playful elements (music) increased adherence to the proposed exercises. Exercise interventions were supervised by a responsible physical therapist; blood pressure and peripheral oxygen saturation were monitored before and after interventions, and heart rate was monitored throughout the exercise.

### **Blood samples and biochemical analyzes**

Blood samples were taken at the pre-intervention moments, 30, 90, 180 and 270 days after starting the intervention. Blood was collected from the antecubital vein of the arm. For analysis of cytokines and leptin, venous blood was collected in two Vacutainer tubes, without anticoagulant (Becton-Dickinson, Oxford, UK). The samples were centrifuged and the serum was aliquoted and frozen at - 800C for further analysis. Systemic levels of leptin, IL-6, IL-10, IL-33 and TNF- $\alpha$  were performed using the Enzyme Linked Immunosorbent method (ELISA) following the manufacturer's recommendations (Peprotech, USA) in an EzReader microplate reader (Biochrom, USA ).

The determination of substances reactive to thiobarbituric acid (TBARS), occurred through the MDA-TBARS technique described by Ohkawa et al (1979). Plasma aliquots (0.2 mL) were added to 250  $\mu$ L of acetic acid (2.5M, pH 3.4) and 250  $\mu$ L thiobarbituric acid (0.8%). This mixture was incubated for 90 minutes at 95 ° C and then centrifuged at 5,000 g for 15 minutes. The supernatant of the reaction was collected and analyzed in the microplate reader at 532 nm. The plasma products of advanced oxidation in plasma (AOPP) were determined spectrophotometrically according to the method

proposed by Witko-Sarsat et al. (1996). Briefly, 1 mL of plasma was diluted (1: 5) in phosphate-saline solution (10 mmol / L, pH 7.4) or chloramine-T standard solution (100 mmol / L) and mixed with 50  $\mu$ L of iodide potassium (1.16ml / L) followed by 100  $\mu$ L of citric acid. The absorbance at 340 nm was determined immediately and the concentrations of AOPPs were expressed in mmol / L of chloramine-T equivalents. For the quantification of plasma nitric oxide levels, the Griess test was used. (Miranda et al, 2001)

### **Data analysis**

Statistical analysis was performed using the SPSS 22.0 statistical program (SPSS Inc., USA). The normality of the variables was assessed using the Shapiro-Wilk test, and the data were presented as mean + - standard deviation. The comparison of variables occurred by analysis of variance (ANOVA) of repeated measures followed by a Bonferroni post-hoc for multiple comparisons. A significance level of  $p < 0.05$  was adopted for all analyzes.

## **RESULTS**

22 patients participated in the training. Blood collection was carried out in 14 individuals with a mean age of  $43.12 \pm 6.25$ , 25% female and 75% male. A reduction in BMI and body mass was observed at 90, 180 and 270 days after intervention when compared to the baseline period ( $p < 0.05$ ).

As shown in Figure 1, no significant differences were found for the results of IL-6 and IL-33. IL-10 levels were increased at 90 ( $p = 0.038$ ), 180 ( $p = 0.033$ ) and 270 ( $p = 0.027$ ) days when compared to baseline. There was a significant reduction in the concentration of TNF-alpha at 180 ( $p = 0.02$ ) and 270 ( $p = 0.001$ ) days in relation to the pre-intervention moment.

There was a reduction of the hormone leptin when compared to the baseline moment, being significant in the moments 90 ( $p = 0.02$ ) and 270 days ( $p = 0.028$ ). Figure 2 shows the concentrations of TBARS at different times of intervention. Significant reductions were found in times 180 ( $p = 0.034$ ) and 270 ( $p = 0.042$ ) days in conjunction with an increase in nitrite levels 270 days

( $p = 0.03$ ) of combined training. No significant differences were identified in the levels of AOPP ( $p > 0.05$ ).

## **DISCUSSION**

The main objective of this study was to evaluate in short, medium and long term the effect of combined training on inflammatory parameters and oxidative damage in individuals with schizophrenia. The main findings of this study demonstrate that the combined training was able to promote a modulation either of the inflammatory state or the oxidative damage marker analyzed.

Although no significant difference was found in the pro-inflammatory cytokines IL-6 and IL-33 in our study, modulation of the interleukin anti-inflammatory cytokine 10 was observed. The pro-inflammatory cytokine interleukin 6 has been correlated with the severity of schizophrenia for inducing an inflammatory environment (Gómez-Rubio and Trapero, 2019). This activity may be related to physical inactivity, obesity and the level of disease impairment. Regular exercise is indicated as a non-medication therapy for and prevention of chronic inflammation in these individuals, because, due to the characteristics of the disease, high levels of IL-6 are associated with the severity of cognitive impairment (Gómez-Rubio e Trapero, 2019 ). The function of IL-10 is to maintain the balance between pro-inflammatory and anti-inflammatory cytokines acting in a regulatory manner. In our study, this interleukin showed a significant increasing and with anti-inflammatory characteristics at all collection times after 90 days (180 and 270 days). We believe that in the previous times (pre and 30) their levels did not increase due to the process of physical adaptation observed during the exercise. In the phase of adaptation to exercise, there is an induction of a natural inflammatory response (Silva and Macedo, 2011) due to the micro lesions that occur in skeletal muscle tissue. After this phase, conditioning occurs, when there is a reversal through the modulation of pro-inflammatory cytokines as observed in our study (Gleeson et al, 2012).

The increase in IL-10 was accompanied by a decrease in TNF- $\alpha$  levels at 180 and 270 days after starting the intervention, confirming an anti-

inflammatory environment. The IL-10 cytokine has its anti-inflammatory action blocking the production of pro-inflammatory cytokines released predominantly by macrophages (Armstrong et al, 1996). The cytokine TNF- $\alpha$  plays an important role in cardiovascular diseases, in the case of schizophrenic individuals there is evidence that demonstrates the higher blood TNF- $\alpha$  levels compared to healthy individuals (Kunz et al, 2011; Lee et al, 2016) . In our study, the reduction of this inflammatory environment can be explained by the combined physical activity protocol, respecting the adaptation time of 3 months.

The cytokine IL-33 belongs to the IL-1 family and is associated with the type 2 helper T cell phenotype (also known as Th2). Its function is the ligand of the ST2 receptor expressed in CD4 + Th2 T lymphocytes, mast cells and type 2 innate lymphoid cells (Miller, 2011). Thus, IL-33 is a cytokine with a potent antiinflammatory and immunosuppression action. Interestingly, IL-33 levels are reduced in patients with psychiatric disorders (de Campos-Carli et al, 2017). So far, very little is known about the effect of physical exercise either on intracellular signaling or on the systemic levels of this cytokine. However, one study showed that combined training raises circulating levels of IL-33 together with an attenuation in the expression of the main intracellular inflammatory transcription factor NF-kappaB and the Toll Type 4 receptor (TLR-4) in cells peripheral of type 2 diabetics (Liu et al, 2015). Likewise, our group identified elevated levels of systemic IL-33 and IL-10 in eutrophic and obese individuals with moderate and high cardiorespiratory capacity compared to sedentary individuals with low cardiorespiratory capacity, showing the important link between improved functional capacity and the elevation of anti-inflammatory cytokines (Dorneles et al, 2019). However, in the present study, it was not possible to identify changes in IL-33 levels.

Exercise is a promising intervention to reduce the deleterious effects of physical inactivity in patients with schizophrenia (Curcic et al, 2017). These effects are directly related to the increased risk of cardiovascular disease, metabolic syndrome, diabetes, among others. In addition, physical exercise is significantly favorable to health, providing psychological benefits and

social integration, improving self-esteem and well-being (Schmitt et al, 2018; Tumiel et al, 2019). The concurrent exercise protocol is composed of resistance and aerobic exercises. As described in the literature, resistance training is able to improve aspects of the physical and mental symptom. By adding aerobic exercise to the protocol, it is possible to improve cognitive aspects and negative symptoms of the disease (Dauwan et al, 2016).

For the leptin results, we found a significant reduction in the times 90 and 270 days after the beginning of the intervention, considering that the majority of the participants were overweight or obese. Leptin is a hormone related to satiety. In the case of obese patients, leptin is increased due to the large number of adipocytes, however it is unable to act in satiety since they become refractory to the action of leptin because it does not reach their receptors (Chan et al, 2002). Thus, the physical exercise protocol used in the study was able to contribute to the improvement of the imbalance condition of this hormone and, together with the reduction of BMI, it seems to help in the control of obesity. Although the literature is conflicting regarding this mechanism, some studies have also found a decrease in leptin associated with loss of body fat in chronic exercise (Perusse et al, 1997). In addition to loss of body fat, Miyatake et al. (Miyatake et al , 2004) observed a decrease in insulin, BMI and leptin after a 1-year exercise program, The interaction between markers of oxidative stress and leptin concentration is observed in overweight and obese patients (Frühbeck et al, 2017; Sobrealeb et al, 2017). In our study, the levels of the TBARS marker showed a significant reduction in 180 and 270 days. TBARS consists of a method of indirect measurement of oxidative stress through the analysis of final lipid peroxidation products that is based on the reaction of malondialdehyde (MDA) with thiobarbituric acid (TBA) (Powers and Jackson, 2008). The numbers found in this test are directly associated with oxidative stress. Based on this and our results, we also observed in this parameter a phase of adaptation of about 3 months. However, in this case, this gradual increasing up to 3 months can be justified by the pre-existing physical and metabolic condition. When exercising acutely, oxidative stress can be significantly increased, but when the exercise is performed in a

chronic way, adaptations and strengthening of the organism as a whole occur. This adaptation can be in the form of a reduction in the levels of oxidative and inflammatory markers ( Angarten et al, 2011).

Our study identified an increase in systemic levels of nitrite, a metabolite of nitric oxide, at the end of the combined training period. Schizophrenic patients have important cardiovascular changes, especially endothelial dysfunctions, which are associated with the condition and worsening the picture of cognitive disorders (Grove et al, 2015). Chronic physical exercise induces an improvement in endothelial function in several conditions associated with health (that is, in sedentary individuals, the elderly, type 2 diabetes and cardiovascular diseases), stimulating an increase in the dilation of blood vessels through an improved signaling of vasodilator molecules such as nitric oxide (Ashor et al, 2015; Seals et al, 2019). However, the effect of combined exercise on endothelial function and its related biochemical markers is still poorly established. Lifestyle modification programs had little effect on nitric oxide concentrations in schizophrenic patients (de-Oliveira et al, 2018). It is possible that an increase in the bioavailability of nitric oxide in the plasma of schizophrenic patients occurs due to an attenuation in the systemic redox imbalance after the combined training period. In fact, there is an inverse relationship between systemic levels of nitrite and lipid peroxidation markers so that the imbalance between these conditions favors a progression in the formation of atherosclerotic plaque (Baskin et al, 2003).

Physical activity inserted in the routine of schizophrenic individuals can promote functional recovery, decrease negative symptoms, cognitive deficits, in addition to mitigating the harmful effects caused by oxidative stress (Schneider and Oliveira, 2004; Maurus et al, 2020).

## **CONCLUSION**

The present study contributes to a better understanding of the role of physical activity for the physical condition of this population. Through this study, exercise is encouraged as a non-pharmacological, low-cost and

simple strategy so that this population can benefit by mitigating the psychosocial and physical effects imposed by this psychiatric condition. Our results demonstrate that combined training can be performed safely in patients with schizophrenia, as it promoted an immunoprotective and immunoregulatory effect dependent on the intervention time, improving the antiinflammatory and oxidative response by reducing specific markers.

### **Study limitation**

Impossibility of progression and individualization of the load used in resistance exercises. Due to the psychological and cognitive aspects of the population studied, it was not possible to perform a variety of exercises and implement more complex activities.

### **Thanks:**

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### **Conflict of interest:**

The authors declare that there is no conflict of interest.

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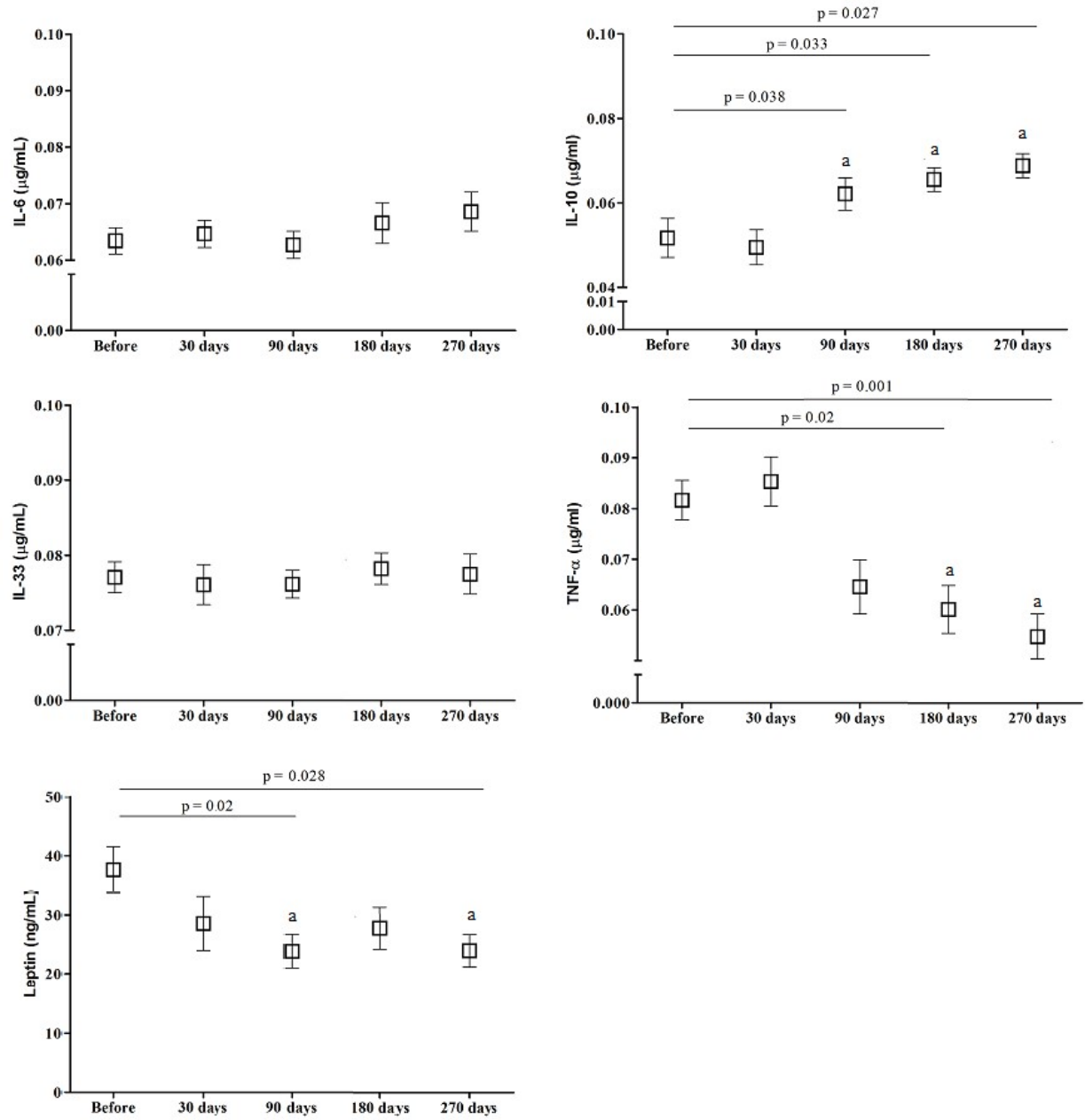
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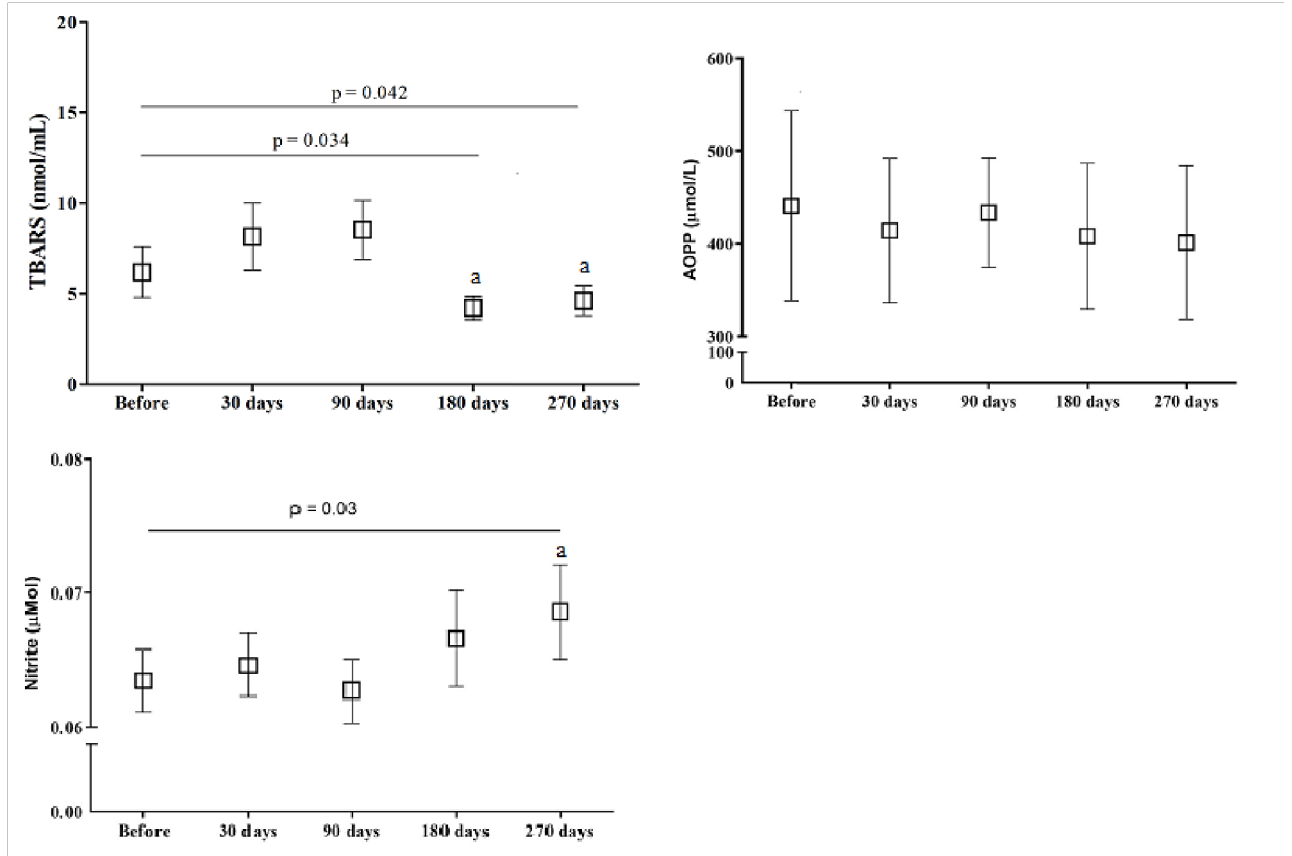
## Figures

**Figure 1: Concentration of cytokines and leptin at the pre, 30, 90, 180 and 270 days after starting the intervention.**



<sup>a</sup> Significant difference when compared to baseline. ( $p < 0.05$ )

**Figure 2: Concentration of TBARS, nitrite and AOPP levels in the pre, 30, 90, 180 and 270 days after starting the intervention.**



<sup>a</sup> Significant difference when compared to baseline. (p < 0.05)

## 5 ARTIGO 3

Artigo em apreciação pela revista Comparative Exercise Physiology. Fator de Impacto: 0.67. Qualis: B4.

### **Time course of epigenetic modulation in response to concurrent exercise training in patients with schizophrenia**

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**Abstract**

This study aimed to investigate the short and long-term effects of concurrent exercise training on anthropometric variables, HDCA2 activity and cortisol levels of individuals with schizophrenia (SZ). Therefore, 10 patients were submitted to the program (endurance and strength exercises in the same session, 60 min of duration, three times a week) and blood samples were collected before, 30 days and 180 days after the intervention started. Exercise training reduced the body mass index and body mass after 180 days of the intervention. A significant decrease on HDAC2 activity was found 180 days after intervention compared to before the intervention. The cortisol levels remained unchanged in any evaluated time-points. The concurrent exercise training was able to modulate HDAC2 activity in mononuclear cells and improve anthropometric variables in a time-dependent manner in patients with SZ.

**Keywords:** schizophrenia; exercise; epigenetic; cortisol.

## 1. Introduction

Schizophrenia (SZ), one of the most debilitating psychiatric disorders, affects approximately 1% of the world's population (Freedman, 2003; Adam 2013). Although antipsychotic medications are able to improve the psychotic symptoms, they are ineffective against cognitive deficits observed in these individuals (Ibrahim and Tamminga 2012; Millan, et.al, 2012). In fact, SZ patients usually show deficits in cognitive domains such as working memory and executive function (Goldberg et al.,1993; Fervaha et al., 2015; Nielsen et al., 2015; Husa et al., 2016). On the other hand, the regular practice of different physical exercise protocols have been pointed out as adjuvant treatment to medication for SZ, showing to be effective in improving negative and general symptoms, cognition, global functioning and quality of life (Lin et al., 2011; Visceglia and Lewis, 2011).

Emerging evidences have been highlighting that the disruption of epigenetic mechanisms might play a pivotal role in the pathophysiology and development of schizophrenia symptoms (Liu et al., 2014; Bahari-Javan et al., 2012; Ibi and Gonzales, 2015; Kebir et al., 2014; Kurita et al., 2012). Epigenetic mechanisms, induced by external environment, are able to alter chromatin structure and modulate gene expression without changes in the DNA primary sequence. DNA methylation and post translational histone modifications such as histone acetylation are the mechanisms responsible for these changes. Histone acetylation, catalyzed by histone acetyltransferases (HATs) enzymes are linked to enhanced transcriptional activity while the opposite is done by histone deacetylases (HDACs), contributing to gene silencing (Kouzarides, 2007).

It is reported that overexpression of HDAC2, a specific class of HDACs, is able to reduce dendritic spine density, synapse number and negatively regulates transcription of genes involved in synaptic plasticity and memory (Kurita et al.,2012; Ibi et al.,2017; Guan et al.,2009). In addition, enhanced levels of HDAC2 has been linked to schizophrenia-like behavior in preclinical studies (Bahari-Javan, et al., 2012; Kurita et al., 2012). Some authors also demonstrated that chronic treatment with atypical antipsychotic drugs, such as clozapine and risperidone, induced an up-regulation of HDAC2 in mice and in postmortem human frontal cortex tissue samples (Ibi et al.,2017; Kurita et al.,2013).

Contrarily, peripheral and local administration of HDAC inhibitors in frontal cortex and nucleus accumbens demonstrated to affect memory function and depression symptoms in animal models (Kurita et al., 2012; Guan et al.,2009; Covington et al.,2009; Graff et al., 2012; Morris, Mahgoub, Na, Pranay and Monteggia,2013). These beneficial effects of HDAC

inhibitors in brain function were also reported in clinical evidences showing cognitive improvements after the intervention (Casey et al., 2009; Meltzer et al., 2011). Taken together, these data support the potential utility of HDAC inhibitors as therapeutics for SZ, suggesting that strategies that modulate this epigenetic marker should be investigated as potential approaches for this population.

In this context, it is well established that diverse exercise protocols can induce epigenetic modifications in different populations (Dorneles et al., 2016; Dorneles et al., 2017; Da Silva et al., 2017; Korbet et al., 2018, Lavratti et al., 2017; Zhang et al., 2015; Oliveira and Iraci et al. 2020). Specifically, Dorneles and colleagues (2017) showed reduced levels of HDAC2 in peripheral blood from obese individuals after an acute strenuous exercise protocol. In addition, we recently demonstrated that a concurrent exercise protocol during 3 months induced global histone H4 hypoacetylation levels and improved anthropometric measurements in SZ patients, suggesting that these individuals are susceptible for epigenetic modulation in response to exercise (Lavratti et al., 2017). However, clinical trials with longer interventions regarding this issue in SZ individuals are not available.

Finally, it is widely reported abnormal Hypothalamic-pituitary-adrenal (HPA) axis functioning in SZ patients (Bradley & Dinan, 2010; Mondelli et al., 2010) a neuroendocrine system that exerts a pivotal role in coordinating the stress response via the secretion of glucocorticoid hormones, mainly cortisol (Gunnar and Quevedo, 2007). Specifically, persistent stress exposure, as it is linked to SZ, seems to lead chronic elevations in cortisol release activity (Walker, Mittal and Tessner, 2008), which contribute to worsen the schizophrenic symptoms, ill-health such as metabolic syndrome and diabetes mellitus and consequently, to poorer quality of life and premature mortality (Bradley and Dinan, 2010; Gury, 2004; Hampel et al., 2010; Walker et al. 2004; Laursen, 2011; Ryan and Thakone, 2002). Furthermore, Kaliman and colleagues (2014) suggested a mechanistic link between cortisol and the peripheral HDAC regulation as well the immune suppressive action of glucocorticoids involves deacetylation of histones via augmentation of HDAC2 activity. However, the link between cortisol and HDAC2 has been not investigated in SZ individuals.

In view of all these considerations, this study aimed to evaluate the effect of a concurrent exercise protocol on anthropometric variables, HDAC2 activity and cortisol levels in peripheral blood of SZ patients in different time-points: before, 30 days and 180 days after the intervention began.

## 2. Material and methods

### 2.1 Subjects

Twenty-two individuals with SZ diagnostic (according to the diagnostic criteria of DSM-V) of both genders aged 18-50 years that participate at the *Associação Gaúcha de Familiares de Pacientes Esquizofrênicos* (AGAFAPE) were recruited. Be in medical treatment and regular use of medication for their illness; not be in a psychotic crisis, not be making use of alcohol and other drugs; not physically active (does not regularly exercise or engage any physical activity program during the past six months) are considered the inclusion criteria to participate. The exclusion criteria were: the presence of autoimmune disease, cancer, cardiovascular complications and musculoskeletal and joint disorders that made it impossible to carry out physical exercise.

This study was approved by the Research Ethics Committee of the *Universidade Federal de Ciências da Saúde de Porto Alegre* (n. 2.464.092) and all experimental procedures were performed in accordance with the Declaration of Helsinki. All participants provided written informed consent prior to participation.

### 2.2 Intervention

The participants were submitted to an intervention characterized by the combination of aerobic and resistance training, named concurrent exercise protocol. The intervention was done in group, during 180 days, 3 times/week, 60 minutes each session, as previously described by Lavratti and colleagues (2017).

Summarizing, the protocol was composed by 30 minutes of aerobic circuits, followed by resistance exercises. Importantly, the recommendations of the American College of Sports Medicine were used during the intervention (American College of Sports Medicine, 2006). In this context, to ensure the safety of participants, all sessions were monitored and supervised by an physical therapist. Also, the participants received constant verbal motivation during the training and were asked to adhere to their regular diet throughout the intervention course (Lavratti et al., 2017).

### 2.3 Experimental Design

In order to evaluate the short- and long-term effects of the concurrent exercise program on the HDAC2 activity and cortisol levels, blood samples were taken (15 ml) in the antecubital region of individuals in 3 times: before the intervention, 30 and 180 days after the intervention started. Subjects were instructed to avoid strenuous physical exertion and alcohol or caffeine in a 24-hour period prior to blood collections.

#### *2.4 Blood collection and sample procedure*

Initially, using the density gradient technique as described by Bicalho et al. (1981) (Bicalho et al., 1981) the peripheral blood mononuclear cells (PBMCs) were isolated. Specifically, whole blood samples were diluted in a proportion of 4:3 in phosphate-buffered saline (PBS, 136 mM, NaCl, 2.7 mM KCl, 7.8 mM Na<sub>2</sub>HPO<sub>4</sub>, 1.7 mM KH<sub>2</sub>PO<sub>4</sub>; pH 7.2-7.4) and centrifuged (1500 rpm, 21° C, 20 min) on Ficoll-Histopaque 1077 (Sigma, MO, USA). Therefore, 1.5 ml of serum was collected, separated and frozen at -20 for circulating cortisol analysis while PBMCs were collected and washed 2 x in PBS (2000 rpm, 21° C, 8 min) and frozen at -80 for HDAC2 measurement.

#### *2.5 HDAC2 activity measurement*

The HDAC2 activity was determined using the EpiQuick HDAC2 Assay Kit (Colorimetric Detection EpiGentek USA) according to the manufacturer's instructions. Results were calculated using a standard curve and expressed as ng/mg of protein. The Coomassie Blue method using bovine serum albumin as standard was used to determine the protein concentration of each sample (Bradford, 1976).

#### *2.6 Cortisol analysis*

The analysis of serum cortisol was performed using competitive electrochemiluminescence immunoassay (Cobas 8000-Roche, Elecsys).

#### *2.7 Statistical Analysis*

After the normality (Shapiro-Wilk) and variance (Levene's) tests, data were considered parametric and presented as mean ± standard deviation. A repeated measure variance analysis was adopted to evaluate the effect of time (baseline, 30 and 180 days). The Bonferroni post-test was used for multiple comparisons. P values ≤ 0.05 were considered statistically significant and SPSS 20.0 (SPSS Inc., Chicago, USA) was used.

### 3. Results

A total of 12 individuals were recruited for the study. During the program, no participants withdrew, and therefore 12 individuals successfully completed the 180 days intervention period. However, 2 patients did not perform blood collection at any of the evaluated times and therefore the results of the biomarkers evaluated are from 10 individuals. The sample characterization is described in Table 1.

The intervention improved the anthropometric data, since a significant reduction on body mass ( $F=70.15$ ;  $p<0.001$ ) and body mass index (BMI)( $F=68.00$ ;  $p<0.001$ ) was observed 180 days when compared to the baseline (Table 1).

Figure 1 highlights the effect of exercise concurrent protocol on HDAC2 activity in PBMC of patients with SZ in different time-points: pre, 30 and 180 days after the intervention started. We observed a significant decrease in this marker 180 days comparing it to 30 days and before intervention ( $F=8.65$ ;  $p=0.027$ ;  $p=0.004$ , respectively). On the other hand, cortisol levels remained unchanged after intervention in all evaluated time-points (Figure 2;  $F=1.12$ ;  $p>0.05$ ).

### 4. Discussion

To our knowledge, this is the first evidence demonstrating that patients with SZ are vulnerable to HDAC2 changes induced by exercise at short and long term. On the other hand, although some evidences have been reported that HDACs can act positively or negatively on inflammation and immune response (Aung et al., 2006; Ito, Barnes and Adcock, 2000), the modulation of this epigenetic signal in the current study was not linked to changes in cortisol levels.

Our results are in agreement to several clinical studies pointing out that physical activity, including acute and chronic protocols, acts as a powerful epigenetic modulator in different populations (Dorneles et al., 2016; Dorneles et al., 2017; Da Silva et al., 2017; Korb et al., 2018, Lavratti et al., 2017; Figueiredo et al., 2017). Specifically, we showed an up-regulation on HDAC2 at short term (30 days after the intervention), an indicative of reduced transcriptional activity and gene expression. In accordance, our group previously demonstrated a significant histone H4 hypoacetylation status in PBMCs following 1 month of a concurrent exercise protocol in SZ individuals (Lavratti et al., 2017).

Collectively, these data raise the possibility that exercise, specifically concurrent protocols, could transcriptionally silence genes that exert a pivotal role in the pathophysiology and progression of SZ through epigenome modulation. This hypothesis could be related, at least in part, to findings obtained by Denham and colleagues (2015) since these authors reported that genes associated with several diseases, including SZ, were hypermethylated after 3 months of aerobic exercise training in sperm of young healthy men (Denham, et al., 2015).

Importantly, we showed that 180 days were able to significantly decrease HDAC2 activity. Our data can be related to those obtained by Dorneles and colleagues (2016) who found an increase of 135% on HDAC activity immediately after a high intensity interval exercise session in overweight-obese individuals and the levels of this enzyme returned to baseline 24 hours later (Dorneles et al., 2016). Furthermore, Korb and colleagues (2018) also reported a similar profile on HDAC activity in response to exercise in diabetes mellitus patients, since exercise performed in both water and dry land environments acutely increased global HDAC activity, while 12 weeks of chronic exercise reduced this parameter (Korb et al., 2018). Taken together, these findings led us to infer that the acute effects of exercise on HDAC activity may be involved in the hematological changes and anti-inflammatory responses following training at short time (Dorneles et al., 2016; Korb et al., 2018). On the other hand, it is possible to suggest that the beneficial effects are related to training-induced adaptations in epigenetic modifications. In addition, more prolonged exercise exposures seem to be protective to SZ individuals, since it was recognized that inhibition of HDAC2 protects against pro-psychotic insults and improves short-term recognition memory in experimental model of SZ (de La Fuente Revenga et al., 2018).

The intervention was not able to change the systemic cortisol levels in SZ patients after 180 days of intervention. This result is in line with our previous study which found no changes in cortisol levels after 90 days of exercise training despite the anti-inflammatory and histone H4 hypoacetylation status of PBMC adaptations in SZ patients (Lavratti et al., 2017). In this sense, six months of Tai Chi Chuan and combined exercise training had little effects on cortisol levels of SZ patients despite improvements of functional capacity (Ho et al., 2016). Possibly, the hyperactivity of HPA axis in people suffering from SZ may create a strong inhibition of HPA negative feedback loop, inducing a resistance to changes in glucocorticoid hormones by exercising (Nugent et al., 2015). On the other hand, Ho and coworkers (2016) revealed that the total amount of daily physical activity behavior was linked

to reduced cortisol levels psychotic symptoms (Ho et al., 2016). In this sense, future studies should evaluate the impact of daily time spent to total physical activity levels, and not only exercise training session, on the regulation of HPA axis and systemic cortisol levels.

Finally, another remarkable point to discuss is the improvement on BMI and body mass following 180 days of intervention, while no changes in any anthropometric variables were observed at short term (3 months). Our results are in accordance to Silva et al. (2015) who demonstrated that a 20-week program of resistance or concurrent exercise did not change anthropometric data in SZ individuals (Silva et al., 2015). Therefore, although it is reported that concurrent training is able to optimize the regulation of glucose metabolism, increase lean body mass, improve body composition, as well as prevent the bone loss in SZ individuals (Meyer and Lehman, 2006; Vancampfort et al., 2012), taken together, these findings suggest that these beneficial effects require more prolonged and chronic interventions.

## **5. Conclusions**

Summarizing, our findings indicate that a concurrent exercise protocol modulates HDAC2 activity and anthropometric variables in a time-dependent manner in SZ individuals. Particularly, the intervention at long term (180 days) may represent a new approach and provide a therapeutic avenue by inducing HDAC2 inhibition/decrease as well significant improvements on body mass and body mass index. Importantly, these responses did not engage changes on cortisol levels.

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## **Conflict of Interest**

The authors declare that they have no conflict of interest.

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### **Figure Legends**

**Figure 1.** The impact of a concurrent exercise protocol on HDAC2 activity in patients with schizophrenia at different time-points.

**Figure 2.** The impact of a concurrent exercise protocol on cortisol levels in patients with schizophrenia at different time-points.

## Tables

**Table 1.** Sample characteristics before and after intervention (n=12)

	Before	30 days	180 days
Gender (male/female)	9/3	-	-
Age (years)	42,41 ± 7,44	-	-
Diagnostic time (years)	24,91 ± 10,60	-	-
Height (meters)	1,72 ± 0,06	-	-
Body mass (kg)	73,91 ± 10,76	72,89 ± 11,28	70,68 ± 10,89*
BMI (kg/m <sup>2</sup> )	24,88 ± 2,89	24,42 ± 3,03	23,78 ± 2,94*

Data presented as mean ± standard deviation (numeric data).

\*Denote statistical differences from before intervention (p<0.05).

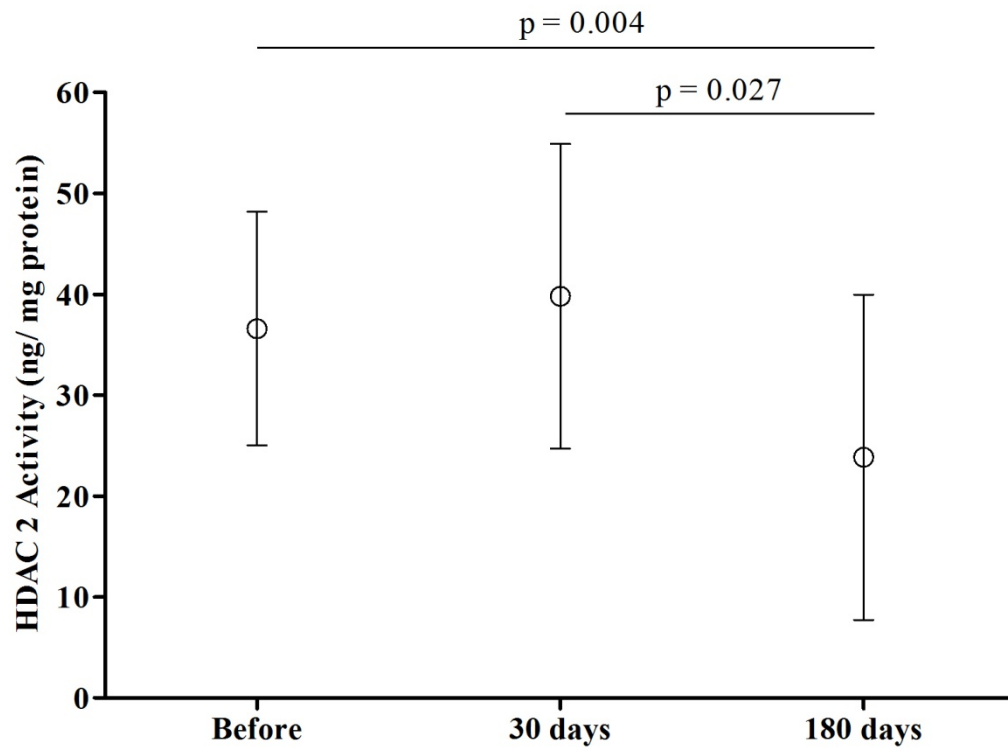
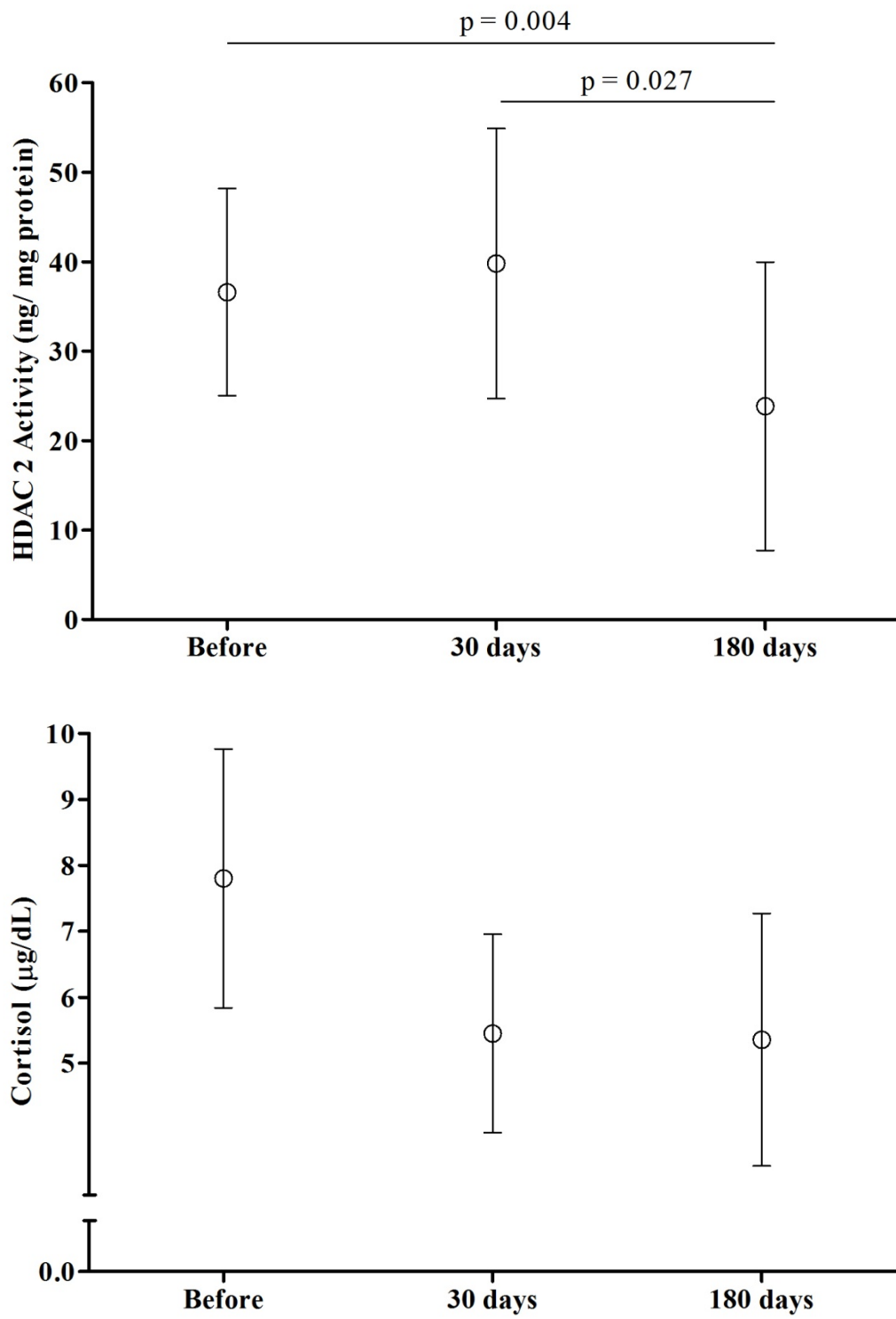
**Figures****Figure 1**

Figure 2



## 6 CONCLUSÃO GERAL

A esquizofrenia está associada a várias comorbidades como doenças cardiovasculares, diabetes e obesidade, decorrentes da condição inflamatória que estes indivíduos apresentam, bem como do isolamento social e da medicação utilizada. Acredita-se que o presente estudo contribuiu de forma significativa na compreensão de que o treinamento combinado é capaz de modular fatores biológicos avaliados através da atividade epigenética (redução da HDAC), do padrão inflamatório (redução da citocina pró-inflamatória TNF alfa e aumento da citocina anti-inflamatória IL-10) e do dano oxidativo (redução de peroxidação lipídica). Foi possível verificar uma melhora nestes marcadores avaliados, indicando que o treinamento combinado é uma excelente estratégia não farmacológica para contribuir na melhoria da saúde da população estudada. Também foi possível verificar uma melhora fisiológica através da redução das medidas antropométricas e também da capacidade funcional (melhora no teste da caminhada, aumento da preensão palmar). Desta forma, exercícios que não requerem grandes espaços e, tão pouco, aparelhos sofisticados, ou seja, atividades de baixo custo e de fácil aplicação são capazes de modular os principais fatores envolvidos tanto com a condição da doença em si quanto com as comorbidades associadas.

Acredita-se que a aplicação do treinamento combinado de forma individualizada e monitorada associada com a realização do mesmo a longo prazo possa apresentar resultados ainda mais significativos pois consideramos como os principais fatores limitantes do estudo a dificuldade em realizar a individualização, a não progressão de carga e a implementação de atividades de maior complexibilidade devido aos aspectos psicológicos e cognitivos da população estudada.

## 7 IMPACTOS DO TRABALHO

Durante séculos, indivíduos com transtornos mentais graves e de longa evolução foram excluídos da vida social em todos os seus âmbitos, sendo privados da participação nas tomadas de decisão sobre assuntos familiares, apresentado dificuldades de frequentar escolas regulares e de compor o mercado do trabalho. Sem dúvidas, esse processo contribuiu para a manutenção de uma vida pouco produtiva, aumentando a chance desses indivíduos se tornarem inativos fisicamente. Além disso, sabe-se que indivíduos sedentários têm maior risco de desenvolver doenças hereditárias e/ou outras condições patológicas, podendo ser físicas, mentais e/ou sociais.

Diante do exposto, o presente trabalho foi capaz de levar o exercício físico e seus benefícios a uma população que ainda sofre com o estigma e com o preconceito, além das limitações acarretadas pela própria condição de saúde. Esse tipo de intervenção promove impactos positivos sobre variáveis fisiológicas, como a atividade cardíaca, a pressão arterial, a frequência respiratória, a temperatura e a atividade muscular. Assim, as atividades propostas por essa pesquisa foram capazes de estimular um estilo de vida mais saudável a esses pacientes, proporcionando, principalmente, a manutenção capacidade funcional. Somado a isso, o exercício físico fornece benefícios psicológicos, proporcionando integração social e melhorando a autoestima e promovendo, conseqüentemente, maior na qualidade de vida.

Por fim, a presente pesquisa apresentou resultados relevantes para a literatura, ao analisar as bases moleculares e sua associação com os resultados fisiológicos observados nos indivíduos com esquizofrenia. Esses resultados podem servir como base para o incentivo à prática de atividade física regular ela população estudada.

## ANEXOS

### ANEXO A - TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

**PROJETO: Avaliação de biomarcadores cardiopulmonares, metabólicos e epigenéticos em indivíduos com esquizofrenia submetidos a um programa de treinamento físico**

Você está sendo convidado (a) a participar da pesquisa acima, cujo objetivo é avaliar o impacto do exercício físico sobre modulação de parâmetros epigenéticos, marcadores inflamatórios, níveis de BDNF, estresse oxidativo, cortisol, bem como analisar o perfil imunológico, bioquímico e capacidade funcional e força muscular periférica, em indivíduos com esquizofrenia.

Caso aceite nosso convite, o tempo em que você ficará à disposição desta pesquisa será de, aproximadamente, 12 meses, o período em que será realizado o programa de atividade física. Este programa será composto por 3 encontros semanais, nas dependências da AGAFAPE – Associação Gaúcha de Pais e Familiares de pacientes Esquizofrênicos, com sessões de aproximadamente 60 minutos, onde serão aplicados diferentes exercícios por um educador físico habilitado.

Você fará dois testes: um para avaliação da força muscular periférica ( será instruído a realizar o máximo de força de preensão possível, apertando com a mão dominante as duas hastes da manopla durante aproximadamente quatro segundos), e o segundo o teste de caminhada de 6 minutos (caminhar em uma pista de 30m durante 6 minutos) para avaliar a capacidade funcional; esses testes serão realizados antes e após a intervenção do programa de atividade física.

Para a realização dos ensaios bioquímicos, será coletado 15 ml de sangue em 5 situações, antes do início da intervenção, 1, 3, 6, 9 e 12 mês após o início da mesma, por um profissional habilitado.

A coleta sanguínea, os testes e as avaliações antropométricas serão realizados nas dependências da AGAFAPE.

Conforme Resolução 466/12 CNS, a qual regulamenta as diretrizes e normas de pesquisas envolvendo seres humanos no Brasil, este projeto apresenta risco moderado para você, uma vez que prevê coletas sanguíneas e que você será submetido a um programa de atividade física de intensidade moderada.

As coletas sanguíneas poderão causar o aparecimento de hematoma temporário e, em alguns casos, pode haver a ocorrência de desmaio. Em ambos os casos, você será atendido e tratado por um profissional devidamente capacitado para resolução do problema no próprio local de coleta (podendo ser um Enfermeiro, Biomédico ou paramédico) para estabelecer seu bem estar físico.

O exercício físico será composto de exercício de força e de resistência. Serão divididos em 30 min de caminhada e 30 min de exercícios resistidos com 3 séries de 8 a 12 repetições, com 8 exercícios envolvendo grandes e pequenos grupamentos musculares.

O exercício físico poderá causar cansaço e desconforto ao participante. Neste caso, você também será atendido, tratado e orientado por um profissional devidamente capacitado que esteja acompanhando o mesmo durante as atividades. Você será orientado a encerrar o treino naquele momento e descansar, sendo que seus sinais vitais serão monitorados. Você será liberado quando se

sentir confortável e estiver com os sinais vitais estáveis. Caso necessário, você será encaminhado a um médico.

Como benefícios à curto prazo, você receberá um relatório com os resultados de todas as suas avaliações e resultados dos exames sanguíneos. Somando-se a isso, a sua participação será importante para o desenvolvimento de novos conhecimentos acerca deste assunto, contribuindo para o crescimento da ciência e oportunizando outros benefícios para a população.

Os seus registros serão sempre tratados confidencialmente. Os resultados deste estudo poderão ser usados para fins científicos, mas você não será identificado (a) por nome.

Sua participação no estudo é voluntária, de forma que, caso você decida não participar, isto não afetará no tratamento normal que você tem direito. Você deverá apresentar uma liberação médica para a participação do mesmo. Você não terá custo nem receberá por participar. As despesas de transporte sua e de seu acompanhante ( caso necessário) será de responsabilidade dos pesquisadores. Sua participação não é obrigatória e, a qualquer momento, você poderá desistir e retirar seu consentimento. Sua recusa não trará nenhum prejuízo em sua relação com o pesquisador(a) ou com a instituição.

Qualquer informação adicional poderá ser obtida através dos telefones (51) 33038804 - Endereço: Rua Sarmento Leite, 245, sala 803, Predio Anexo III, – Centro Histórico – Porto alegre, (54) 91757078 – celular da pesquisadora Caroline Dallazen Lavratti ou (51) 999619331– celular da professora.

Lembramos que você tem a garantia de receber resposta a qualquer pergunta ou esclarecimento a qualquer dúvida acerca dos procedimentos, riscos, benefícios e outros assuntos relacionados à pesquisa, também da liberdade de retirar seu consentimento a qualquer momento e por qualquer motivo e deixar de participar do estudo sem que isto acarrete prejuízo à minha pessoa. Além disso, será assegurada a garantia de que você não será identificado, quando da divulgação dos resultados e que as informações obtidas serão utilizadas apenas para fins científicos vinculados ao presente projeto de pesquisa. Por fim, você não terá nenhum gasto ao aceitar participar da pesquisa.

Assinatura do representante legal: \_\_\_\_\_

Assinatura do participante da pesquisa: \_\_\_\_\_

Assinatura do professor orientador: \_\_\_\_\_

Data: \_\_\_\_\_

**Observação:** O presente documento baseado no item IV das Diretrizes e Normas Regulamentadoras para a pesquisa em saúde, do Conselho Nacional de Saúde (Resolução 466/2012), será assinado em suas vias, de igual teor, ficando uma via em poder do paciente ou de seu representante legal e outra com o pesquisador responsável.

## APÊNDICES

### APÊNDICE A - PARECER CONSUBSTANCIADO DO CEP

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#### PARECER CONSUBSTANCIADO DO CEP

##### DADOS DO PROJETO DE PESQUISA

**Título da Pesquisa:** AVALIAÇÃO DE BIOMARCADORES CARDIOPULMONARES E METABÓLICOS EM INDIVÍDUOS COM ESQUIZOFRENIA SUBMETIDOS A UM PROGRAMA DE TREINAMENTO FÍSICO

**Pesquisador:** Alessandra Peres

**Área Temática:**

**Versão:** 2

**CAAE:** 78399317.8.0000.5345

**Instituição Proponente:** Universidade Federal de Ciências da Saúde de Porto Alegre

**Patrocinador Principal:** Financiamento Próprio

##### DADOS DO PARECER

**Número do Parecer:** 2.464.092

##### Apresentação do Projeto:

A Esquizofrenia, foco do presente estudo, é uma perturbação que dura pelo menos seis meses e inclui pelo menos um mês de fase e sintomas ativos. Recentemente, a fisiopatologia da esquizofrenia tem sido associada com o desequilíbrio de marcadores inflamatórios e parâmetros epigenéticos, bem como reduzidos níveis do fator neurotrófico derivado do encéfalo (BDNF) e alterações nos níveis de cortisol. Desta forma, estratégias que modulem estes parâmetros parecem ser importantes ferramentas tanto preventivas quanto terapêuticas para o manejo desta população. Neste contexto, destaca-se o exercício físico, caracterizado pelo baixo custo e fácil acesso, uma importante estratégia que tem demonstrado apresentar efeitos antidepressivos, além de melhorar a autoestima e reduzir os níveis de estresse em diferentes populações. Apesar de inúmeras evidências pré-clínicas e clínicas demonstrarem que a atividade física aumenta os níveis centrais e periféricos de BDNF, bem como altera mecanismos epigenéticos e inflamatórios, o efeito de sua prática sobre estes marcadores em pacientes com transtornos mentais graves e de longa evolução tem sido pouco explorado. Desta forma, este estudo tem como objetivo avaliar o efeito de um protocolo de treinamento concorrente de 40 semanas, com três sessões semanais com duração de 60 minutos por dia (dividida em 30 min de caminhada e 30 min de exercícios de força, as quais serão incrementadas ao longo do tempo), sobre dados antropométricos, Marcadores epigenéticos, níveis de BDNF, citocinas inflamatórias.

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Continuação do Parecer: 2.464/202

cortisol, citocinas, bem como o perfil celular imunológico e bioquímico e marcadores de estresse oxidativo e marcadores de função endotelial bem como capacidade funcional em indivíduos com esquizofrenia. Afim de se avaliar os efeitos agudos e tardios do exercício físico sobre estes parâmetros, serão realizadas coletas sanguíneas em 6 momentos: antes do início da intervenção, 1,3,6,9 e 10 meses após seu início

**Objetivo da Pesquisa:**

**Objetivo Primário:**

O foco do projeto consiste em avaliar o impacto do exercício físico sobre a modulação de parâmetros epigenéticos, marcadores inflamatórios, níveis de BDNF, estresse oxidativo, cortisol, bem como analisar o perfil imunológico, bioquímico e capacidade funcional e força muscular periférica, em pacientes com transtornos mentais graves e de longa evolução.

**Objetivo Secundário:**

- Avaliar os níveis de citocinas anti e pró inflamatórias antes do início do programa, 1, 3, 6, 9 e 12 meses após um programa de atividade física em indivíduos esquizofrênicos.
- Avaliar a modulação de marcadores de estresse oxidativo antes do início do programa, 1, 3, 6, 9 e 12 meses após um programa de atividade física em indivíduos esquizofrênicos.
- Quantificar os marcadores de função endotelial: fator de crescimento endotelial vascular (VEGF), óxido nítrico, MCP-1, TNF- $\alpha$ , antes do início do programa, 1,3,6, 9 e 12 meses após um programa de atividade física em leucócitos destes indivíduos esquizofrênicos.
- Avaliar a capacidade funcional dos pacientes, 1, 3,6, 9 e 12 meses após um programa de atividade física em indivíduos esquizofrênicos.
- Avaliação da força muscular periférica, 1, 3,6, 9 e 12 meses após um programa de atividade física em indivíduos esquizofrênicos.
- Quantificar os níveis globais de metilação de DNA antes do início do programa, 1, 3,6, 9 e 12 meses após um programa de atividade física em leucócitos destes indivíduos esquizofrênicos.
- Quantificar a atividade das enzimas DNMT1 e DNMT3b antes do início do programa, 1, 3,6, 9 e 12 meses após um programa de atividade física em leucócitos destes indivíduos esquizofrênicos.
- Quantificar os níveis globais de acetilação da histona H3 antes do início do programa, 1, 3,6, 9 e 12 meses após um programa de atividade física em leucócitos destes indivíduos esquizofrênicos.
- Quantificar a atividade das enzimas HAT e HDAC antes do início do programa, 1, 3,6, 9 e 12 meses após um programa de atividade física em leucócitos destes indivíduos esquizofrênicos.
- Avaliar o perfil imunológico das células T auxiliares (CD3+CD4), T citotóxicas (CD3+CD8), NK (CD3-CD16+CD56+) monócitos (CD14+) e T reguladoras (CD4+CD25+FOXP3+)

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cortisol, citocinas, bem como o perfil celular imunológico e bioquímico e marcadores de estresse oxidativo e marcadores de função endotelial bem como capacidade funcional em indivíduos com esquizofrenia. Afim de se avaliar os efeitos agudos e tardios do exercício físico sobre estes parâmetros, serão realizadas coletas sanguíneas em 6 momentos: antes do início da intervenção, 1,3,6,9 e 10 meses após seu início

**Objetivo da Pesquisa:**

**Objetivo Primário:**

O foco do projeto consiste em avaliar o impacto do exercício físico sobre a modulação de parâmetros epigenéticos, marcadores inflamatórios, níveis de BDNF, estresse oxidativo, cortisol, bem como analisar o perfil imunológico, bioquímico e capacidade funcional e força muscular periférica, em pacientes com transtornos mentais graves e de longa evolução.

**Objetivo Secundário:**

- Avaliar os níveis de citocinas anti e pró inflamatórias antes do início do programa, 1, 3, 6, 9 e 12 meses após um programa de atividade física em indivíduos esquizofrênicos.
- Avaliar a modulação de marcadores de estresse oxidativo antes do início do programa, 1, 3, 6, 9 e 12 meses após um programa de atividade física em indivíduos esquizofrênicos.
- Quantificar os marcadores de função endotelial: fator de crescimento endotelial vascular (VEGF), óxido nítrico, MCP-1, TNF- $\alpha$ , antes do início do programa, 1,3,6, 9 e 12 meses após um programa de atividade física em leucócitos destes indivíduos esquizofrênicos.
- Avaliar a capacidade funcional dos pacientes, 1, 3,6, 9 e 12 meses após um programa de atividade física em indivíduos esquizofrênicos.
- Avaliação da força muscular periférica, 1, 3,6, 9 e 12 meses após um programa de atividade física em indivíduos esquizofrênicos.
- Quantificar os níveis globais de metilação de DNA antes do início do programa, 1, 3,6, 9 e 12 meses após um programa de atividade física em leucócitos destes indivíduos esquizofrênicos.
- Quantificar a atividade das enzimas DNMT1 e DNMT3b antes do início do programa, 1, 3,6, 9 e 12 meses após um programa de atividade física em leucócitos destes indivíduos esquizofrênicos.
- Quantificar os níveis globais de acetilação da histona H3 antes do início do programa, 1, 3,6, 9 e 12 meses após um programa de atividade física em leucócitos destes indivíduos esquizofrênicos.
- Quantificar a atividade das enzimas HAT e HDAC antes do início do programa, 1, 3,6, 9 e 12 meses após um programa de atividade física em leucócitos destes indivíduos esquizofrênicos.
- Avaliar o perfil imunológico das células T auxiliares (CD3+CD4), T citotóxicas (CD3+CD8), NK (CD3-CD16+CD56+) monócitos (CD14+) e T reguladoras (CD4+CD25+FOXP3+)

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integração e troca de experiências dos indivíduos durante as sessões pode reverter à tendência ao isolamento e os sintomas depressivos. Todos esses aspectos podem contribuir para a melhora da sua qualidade de vida, encorajando assim, a população não engajada em nenhum tipo de atividade física buscarem esta prática.

**Comentários e Considerações sobre a Pesquisa:**

Os ajustes solicitados (explicações relacionadas ao treinamento físico; rever a atribuição de risco; atribuições dos membros da equipe; ajustes no TCLE) foram realizados.

**Considerações sobre os Termos de apresentação obrigatória:**

Os pesquisadores adequaram o termo de autorização institucional conforme solicitado.

Folha de rosto e termo de anuência do laboratório de imunologia celular sala 803 estão adequados.

Foi elaborado um TCLE para o responsável legal e outro para o voluntário conforme solicitado.

**Recomendações:**

Uma vez que o TCLE destinado ao participante da pesquisa inclui espaço para assinatura do representante Legal torna-se desnecessário um outro TCLE. No entanto, atentar para que ambas assinaturas estejam presentes em todos os documentos.

**Conclusões ou Pendências e Lista de Inadequações:**

Projeto aprovado.

**Considerações Finais a critério do CEP:**

De acordo com Parecer do Relator.

**Este parecer foi elaborado baseado nos documentos abaixo relacionados:**

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_884806.pdf	13/11/2017 11:41:35		Acaito
Declaração de Instituição e Infraestrutura	TAI_atualizada.pdf	13/11/2017 11:38:53	Alessandra Peres	Acaito
Recurso Anexado pelo Pesquisador	carta_resposta.doc	13/11/2017 11:38:35	Alessandra Peres	Acaito
Outros	TCLE_voluntarios_final.docx	13/11/2017 11:38:13	Alessandra Peres	Acaito
TCLE / Termos de Assentimento /	TCLE_representantes_legais_atualizado.docx	13/11/2017 11:38:45	Alessandra Peres	Acaito

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