UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"

RODOLFO AUGUSTO TRAVAGIN MIRANDA

EFEITOS DO TREINAMENTO FÍSICO SOBRE BIOMARCADORES NA SÍNDROME METABÓLICA

Um ensaio clínico controlado

Uma revisão sistemática e meta-análise

Presidente Prudente

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Dissertação apresentada à Faculdade de Ciências e Tecnologia – FCT/UNESP – Campus de Presidente Prudente, para a obtenção do título de Mestre no Programa de Pós-Graduação Stricto Sensu em Fisioterapia.

Orientador: Prof. Dr. Jayme Netto Júnior.

Presidente Prudente
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UNIVERSIDADE ESTADUAL PAULISTA

Câmpus de Presidente Prudente

ATA DA DEFESA PÚBLICA DA DISSERTAÇÃO DE MESTRADO DE RODOLFO AUGUSTO TRAVAGIN MIRANDA, DISCENTE DO PROGRAMA DE PÓS-GRADUAÇÃO EM FISIOTERAPIA, DA FACULDADE DE CIÊNCIAS E TECNOLOGIA - CÂMPUS DE PRESIDENTE PRUDENTE.

Aos 23 dias do mês de março do ano de 2017, às 14:00 horas, no(a) Anfiteatro II, reuniu-se a Comissão Examinadora da Defesa Pública, composta pelos seguintes membros: Prof. Dr. JAYME NETTO JUNIOR - Orientador(a) do(a) Departamento de Fisioterapia / Faculdade de Ciencias e Tecnologia de Presidente Prudente, Prof. Dr. LUIZ CARLOS MARQUES VANDERLEI do(a) Departamento de Fisioterapia / Faculdade de Ciências e Tecnologia, UNESP/Presidente Prudente, Prof. Dr. FABIO DO NASCIMENTO BASTOS do(a) Departamento de Ciências Patológicas / Universidade Estadual de Londrina, sob a presidência do primeiro, a fim de proceder a arguição pública da DISSERTAÇÃO DE MESTRADO de RODOLFO AUGUSTO TRAVAGIN MIRANDA, intitulada **EFEITOS DO TREINAMENTO FÍSICO SOBRE BIOMARCADORES NA SÍNDROME METABÓLICA**. Após a exposição, o discente foi arguido oralmente pelos membros da Comissão Examinadora, tendo recebido o conceito final: Aprovado. Nada mais havendo, foi lavrada a presente ata, que após lida e aprovada, foi assinada pelos membros da Comissão Examinadora.

Prof. Dr. JAYME NETTO JUNIOR

Prof. Dr. LUIZ CARLOS MARQUES VANDERLEI

Prof. Dr. FABIO DO NASCIMENTO BASTOS

Dedicatória

Dedico este trabalho aos meus pais, Ricardo e Maria

Inês, e ao meu irmão Vitor R. T. Miranda.

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O grande sentimento que fica é Gratidão.

SUMÁRIO

APRESENTAÇÃO

Esta dissertação está apresentada de acordo com as normas do modelo alternativo de dissertação do Programa de Pós-Graduação Stricto Sensu em Fisioterapia da Faculdade de Ciências e Tecnologia – FCT/UNESP – Campus de Presidente Prudente. O conteúdo do presente trabalho origina-se a partir da pesquisa entitulada "Efeitos do treinamento físico sobre biomarcadores na síndrome metabólica" que foi conduzida em duas etapas:

I – Ensaio clínico controlado, conduzido no Laboratório de Fisioterapia Desportiva (LAFIDE) na Faculdade de Ciências e Tecnologias FCT/UNESP, campus de Presidente Prudente.

II - Revisão sistemática e meta-análise conduzida pelo aluno durante o segundo ano de mestrado stricto sensu.

Portanto, o presente material está dividido nas seguintes sessões:

- **Introdução** – para contextualização do tema.
- **Artigo 1** - A new approach of aerobic interval training improves chronic low-grade inflammation and quality of life in metabolic syndrome.
- **Artigo 2** – Aerobic versus resistance training on inflammation status among patients with cardiometabolic disease: Systematic review and meta-analysis. Em revisão pelo periódico ***Journal of Science and Medicine in Sport***.
- **Referências** – utilizadas no texto de introdução ao tema.
- **Conclusões** – a partir de ambas as pesquisas realizadas

Ressalta-se que o artigo submetido ao *Journal of Science and Medicine in Sport* está apresentado de acordo com as normas do respectivo periódico.

RESUMO

Introdução: Pesquisas no âmbito de saúde recomendam a prática regular de exercício físico para o tratamento e prevenção de doenças crônico-degenerativas, com destaque para Síndrome Metabólica (Smet), um conjunto de fatores de risco para doenças cardiovasculares e diabetes mellitus tipo II. **Objetivos:** Investigar, por meio de ensaio clínico, os efeitos de 16 semanas de uma nova abordagem de treinamento aeróbio intervalado (TAI) sobre biomarcadores sanguíneos e qualidade de vida em sujeitos com Smet; bem como, por meio de revisão sistemática e meta-análise, comparar os efeitos do treinamento aeróbio *versus* treinamento resistido sobre concentrações séricas de proteína C reativa (PCR), fator de necrose tumoral alfa e interleucina-6 em população cardiometabólica. **Métodos:** Com relação ao ensaio clínico, 36 participantes diagnosticados com Smet foram alocados em grupo TAI (n=19) e grupo controle (n=17). O treinamento foi realizado três vezes por semana, durante 16 semanas, em três diferentes intensidades de trabalho: 5 semanas de intensidade leve, 4 semanas de intensidade moderada, e 4 semanas de alta intensidade. Qualidade de vida e um conjunto de biomarcadores sanguíneos foram avaliados pré e pós intervenção. Com relação à revisão sistemática, estudos foram selecionados após busca eletrônica em três bases de dados (PubMed/MEDLINE, EMBASE e Cochrane Central Register of Controlled Trials – CENTRAL) desde a data mais antiga de publicação até o dia 7 de julho de 2016. Apenas ensaios clínicos randomizados que comparasse treinamento aeróbio *versus* treinamento resistido sobre concentrações séricas de PCR foram incluídos. Além disso, era necessário que os estudos utilizassem uma população cardiometabólica, isto é, pacientes com sobrepeso, obesidade, diabetes tipo 2 ou síndrome metabólica. **Resultados e conclusões:** A nova abordagem do TAI mostrou-se eficaz em reduzir níveis de inflamação crônica de baixo grau (PCR), bem como em aumentar a qualidade de vida, apenas sobre os aspectos físicos, de seus praticantes sem promover sobrecarga à nivel molecular. Ainda, ao compararmos os efeitos do treinamento aeróbio *versus* treinamento resistido, por meio de meta-análise, o treinamento aeróbio demonstrou superioridade em melhorar o perfil inflamatório (fator de necrose tumoral alfa); no entanto, respostas similares foram observadas sobre os desfechos PCR e interleucina-6.

Palavras-chaves: Exercício; Síndrome X Metabólica; Inflamação; Qualidade de vida.

ABSTRACT

Introduction: Researches in the health context recommend regular exercise training for the treatment and prevention of chronic diseases, especially Metabolic Syndrome (MetS), a cluster of risk factors for cardiovascular diseases and type II diabetes. **Objectives:** To investigate, through a clinical trial, the effects of 16 weeks of a new approach of aerobic interval training (AIT) on blood biomarkers and quality of life in subjects with MetS; as well as, through a systematic review and meta-analysis, to compare the effects of aerobic training versus resistance training on serum concentrations of C-reactive protein (CRP), tumor necrosis factor alpha and interleukin-6 in cardiometabolic population. **Methods:** Regarding the clinical trial, 36 participants diagnosed with MetS were allocated to the AIT group (n = 19) and the control group (n = 17). The exercise training was performed three times per week for 16 weeks, at three different workloads: 5 weeks on light intensity, four weeks on moderate intensity, and four weeks on high intensities. Quality of life and a set of blood biomarkers were measured before and after intervention. Regarding the systematic review, studies were selected after electronic search in three databases (PubMed / MEDLINE, EMBASE and Cochrane Central Register of Controlled Trials - CENTRAL) from the earliest date of publication until July 7, 2016. Only randomized controlled trials comparing aerobic versus resistance training on serum concentrations of CRP were included. Furthermore, studies were required to have a cardiometabolic population, that is, patients with overweight, obesity, type 2 diabetes or metabolic syndrome. **Results and conclusions:** The new approach of AIT improved chronic low-grade inflammation (CRP), as well as improving the quality of life of its practitioners without promoting overload at the molecular level. In additoin, when comparing the effects of aerobic training versus resistance training, through meta-analysis, aerobic training demonstrated superiority on decreasing the inflammatory profile (tumor necrosis factor alpha); however, similar responses were observed to CRP and interleukin-6 outcomes.

Keywords: Exercise; Metabolic Syndrome X; Inflammation, Quality of life.

Introdução

Doenças crônicas, exercício físico e marcadores biológicos

A compreensão de processos patológicos que contribuem para o desenvolvimento de doenças crônico-degenerativas tem sido um foco de pesquisas atuais[1,2]. O binômio inatividade física e obesidade[3] é hoje evidenciado como principal contribuinte para doenças de alta prevalência (diabetes e infarto agudo do miocárdio), o que consequentemente aumenta o risco de morte prematura sobre a população[4].

Dentre as doenças crônicas de grande impacto, a síndrome metabólica (Smet) destaca-se por ser considerada um conjunto de fatores cardiometabólicos que aumenta o risco de desenvolver doenças cardiovasculares (DCV) e diabetes tipo II[4,5]. Os componentes da Smet apresentados pela *International Diabetes Federation* (IDF) são atualmente compostos por alterações na pressão arterial, glicemia de jejum, perfil lipídico e obesidade abdominal[5].

É relevante apontar que, fatores exógenos como o nível de atividade física[6] e dieta[7] (alta ingestão calórica e consumo alcoólico) estão diretamente relacionados ao desenvolvimento de desordens metabólicas. Além disso, é evidenciado na literatura que o sedentarismo atua como principal contribuinte para tais desordens, uma vez que o baixo gasto calórico pode levar à obesidade abdominal[6].

Neste sentido, a hipertrofia que ocorre nos adipócitos decorrente do processo de obesidade estimula a infiltração de células do sistema imune, de maneira local, resultando em produção de citocinas pró-inflamatórias, tais como interleucina-6 (IL-6) e fator de necrose tumoral (TNF-α), as quais possuem alcance sistêmico[8]. Desta forma, há comumente nestes sujeitos um aumento brando na concentração plasmática de proteínas

inflamatórias, especialmente de proteína C reativa (PCR), porém, de maneira crônica. À este processo, dá-se o nome de inflamação crônica de baixo grau[9].

Evidências sugerem que o desenvolvimento de doenças crônicas pode estar associado ao processo de inflamação crônica[10-12], comumente avaliada por meio de concentrações séricas de PCR, considerado um marcador plasmático de fase aguda e preditor para eventos cardiovasculares[13].

Mediante o caráter sistêmico da inflamação crônica de baixo grau, a mesma desencadeia diferentes desordens de acordo com o tecido e sistema envolvido. Neste sentido, a liberação de proteínas pro-inflamatórias pelo tecido adiposo contribui para o desenvolvimento da resistência insulínica e diabetes tipo II[14], enquanto que, respostas inflamatórias sobre o sistema nervoso pode contribuir para a ocorrência de doenças neurodegenerativas, como Parkinson e Alzheimer[15]. Além disso, aumentos locais e sistêmicos de citocinas pró-inflamatórias estão relacionadas com o desenvolvimento de câncer de cólon e mama[16].

Além da presença de fatores de risco cardiovasculares e inflamação, alterações plasmáticas sobre diferentes marcadores de função e dano celular estão associados à Smet. Sobrecarga renal, dano hepático (alteração sobre enzimas de dano hepático) e estresse oxidativo compõe algumas condições que merecem atenção[17-20].

A partir do exposto, torna-se evidente a contribuição de processos deletérios para o desenvolvimento de desordens metabólicas, tornando-se necessário medidas que possam prevenir ou atenuar a progressão das mesmas. Desta forma, programas de treinamento físico têm demonstrado ser uma estratégia efetiva e de baixo custo para tal finalidade. Nesse sentido, a prática regular do exercício físico vem sendo utilizada como parte integrativa ao tratamento de doenças crônico-degenerativas, com importantes

benefícios sobre os aspectos de força muscular, capacidade cardiorrespiratória e fatores de risco cardiometabólicos após diferentes tipos de treinamento[20-22].

Dentre os métodos de maior utilização, no âmbito terapêutico, pode-se citar o treinamento resistido (TR) e modalidades de treinamento aeróbio, tais como Moderate-Intensity Continuous Training (MICT), High Intensity Interval Training (HIIT) e Sprint Interval Training (SIT)[20,23]. Embora a recomendação tradicional de exercício físico seja de no mínimo 150 minutos semanais[24] (entre 30 à 60 min de intensidade moderada, 5 vezes por semana), recomenda-se volumes entre 200 e 300 min semanais, afim de obter-se melhores resultados para o tratamento da obesidade[25].

No entanto, ensaios utilizando exercícios aeróbios de alta intensidade, e de curta duração, têm apresentado ganhos similares e até superiores ao MICT[26]. No estudo de Gillen *et al*[23] compararam os efeitos de 12 semanas de SIT (3 x 20 segundos; intensidade supra máxima; duração de aproximadamente 10 min/sessão) versus MICT (aproximadamente 70% FC_{max}, duração média de 50 min/sessão) em homens sedentários (IMC = 26±6kg/m^2). Como resultado, os grupos apresentaram melhora de mesma extensão sobre sensibilidade insulínica, $VO2_{pico}$ e conteúdo mitocondrial no músculoesquelético.

No mesmo sentido, Weston *et al*[20] verificaram superioridade, estatisticamente significante, do HIIT (aproximadamente o dobro de ganho) quando comparado ao MICT sobre capacidade cardiorrespiratória ($VO2_{pico}$) em indivíduos não saudáveis – obesidade, diabetes, Smet e outras. Destaca-se ainda que o protocolo de HIIT mais utilizado era caracterizado por 4 séries de 4 minutos entre 85-95% FC_{pico}, e recuperação ativa de 3 minutos entre 60-70% FC_{pico} (aproximadamente 25 min/sessão). Desta maneira, evidencia-se treinamentos aeróbicos de alta intensidade como uma estratégia tempo-

eficiente para controle e melhora do estado de saúde, o que pode ser transposto para prevenção das doenças crônicas.

Com relação à utilização do TR, em um amplo estudo de revisão sistemática com meta-análise, Lemes *et al*[22] evidenciaram sua eficiência em reduzir a pressão arterial sistólica em aproximadamente 4.1 mm Hg em indivíduos com Smet. No entanto, o TR não alterou significativamente as variáveis de circunferência abdominal, perfil lipídico e glicose em jejum, quando comparado ao grupo controle. Os autores ainda concluem que o TR é um método efetivo para prevenção e tratamento de doenças cardiovasculares, além de ser um método de baixo custo quando comparado à outros procedimentos.

Mediante às características e rotas metabólicas predominantes em diferentes métodos de exercício, Yang *et al*[27] compararam os efeitos do treinamento aeróbio *versus* treinamento resistido sobre hemoglobina glicada em diabéticos tipo II. Embora a comparação tenha se apresentado favorável ao treinamento aeróbio nesta meta-análise (Mean Difference = 0.18, 95% CI [0.01, 0.36]), os autores relatam que esta diferença não assegura uma relevância clínica que sustente o predomínio do exercício aeróbio para o controle glicêmico.

A partir do exposto, nota-se relevante a tríade doente metabólico, marcadores biológicos e exercício físico para melhor compreender o desenvolvimento e propor tratamentos para as doenças crônicas. Vale ressaltar que, embora os métodos de treinamento resistido e aeróbio estejam amplamente abordados na literatura, os mesmos ainda merecem investigação sobre efeitos moleculares e anti-inflamatórios, afim de compreender mecanismos e métodos mais efetivos para o controle da inflamação crônica de baixo grau e, consequentemente, prevenir desordens à ela relacionada.

Artigo 1

A new approach of aerobic interval training improves chronic low-grade inflammation and quality of life in metabolic syndrome

Abstract

Background: Traditional protocols of aerobic interval training (AIT), such as 4 x 4 minutes of intervals at high intensity, has been used and suggested to treat metabolic disorders. However, we proposed a new approach of AIT characterized by three different periods (light, moderate and high intensities), which may be ideal to sedentary subjects with metabolic impairments. **Aim:** the present study aimed to investigate the effects of 16 weeks of a new approach of AIT on clinical biomarkers and quality of life in subjects with metabolic syndrome. **Methods:** Thirty six inactive untrained men and women diagnosed with metabolic syndrome, according to the international diabetes federation criteria, were allocated to AIT group (n = 19) and control group (n = 17). Exercise training was performed three times per week for 16 weeks, and at three different workloads: 5 weeks on light intensity, four weeks on moderate intensity, and four weeks on high intensities. Quality of life and a set of clinical biomarkers (high-sensitivity cardiac troponin t, homocysteine, C-reactive protein, Creatine kinase and fractions, aspartate aminotransferase, alanine aminotransferase creatinine and complete blood count) was measured before and after intervention. **Results:** Body weight was signficantly increased in control group (81.99±15.10 to 83.21±15.13; 95% CI: 0.07; 2.45), whereas in the AIT remained unchanged (97.83±16.48 to 97.23±16.88; 95% CI: -1.75; 0.47). C-reactive protein was reduced after AIT by 36.3% (6.0±4.0 to 3.82±3.43; 95%CI: -3.94; -1.01) and no change was observed in control group (6.3±4.6 to 4.9±4.4, 95%CI: -2.70; 0.41). All the remaining biomarkers did not present changes, except for creatine kinase, which was reduced in both groups. Quality of life was improved only in domains related to the physical aspect, while mental aspects remained unchanged. **Conclusions:** The new approach of AIT improved low-grade inflammation and quality of life among metabolic syndrome. Additionally, our findings suggests that such training program did not present detrimental effects related to cell damage markers.

Keywords: exercise; metabolic syndrome x; inflammatory response.

BACKGROUND

Metabolic syndrome is a cluster of risk factors for cardiovascular diseases (CVD) and type 2 diabetes[1], which consequently increase the risk of premature death[2]. Besides the traditional metabolic risk factors (central obesity, dyslipidemia, raised blood pressure and dysglycemia), subclinical disorders have been demonstrated in metabolic syndrome, including chronic low-grade inflammation[3-5], non-alcoholic fatty liver disease[6] and hyperhomocysteinemia[7].

Studies have highlighted the relationship between low-grade inflammation and the development of atherosclerosis, diabetes and metabolic syndrome[8, 9, 5]. The detrimental effects of the low-grade inflammation differs between tissues and involves plaque formation, insulin resistance and dysregulation of skeletal muscle metabolism[10, 11].

As a traditional marker of inflammation status, C- reactive protein (CRP) is physiologically augmented to defense against injury and infection. However, subjects with metabolic syndrome may have CRP levels naturally elevated, which increases the risk of developing CVD and others inflammation-related diseases[12, 3]. In the same direction, high levels of homocysteine (Hcy), a sulfor-containing amino acid, is reported as a novel CVD risk factor, especially by promoting plaque formation in the endothelium[13]. Humphrey *et al*. demonstrated that each additional 5 μmol/L of homocysteine may increase by approximately 20% the risk of coronary heart disease[14].

On the other hand, significant reduction have been observed after exercise interventions in CRP, Hcy and liver damage enzymes[15, 13, 16]. Thus, given the relevance between biomarkers and diseases development, recent studies have been focused on evaluating biomarkers responses after both acute and chronic exercise training[4,13].

The published literature indicated that aerobic training is an effective and low cost method to improve healthy status regarding metabolic (glycemic and lipid profiles) and anti-inflammatory responses[17, 18]. However, although the aerobic moderate-intensity continuous training is a classical treatment to improve cardiometabolic disorders[19], high-intensity interval training (HIIT) has been shown to elicit comparable or greater improvements in peak oxygen uptake, glycemia control and metabolic risk factors[20-22]. In addition, the most used HIIT protocol is characterized by 4 x 4 min of intervals at 85-95% of peak heart rate (HR_{peak}), with a 3-min of recovery at 70% of HR_{peak} between each interval[23, 21, 4].

Although recent studies have demonstrated HIIT as a time-efficient strategy[24], such protocols do not report adaptative periods (with low workloads), which may be required to minimize metabolic and musculoskeletal overload of untrained and unhealth subjects. Therefore, it appears relevant to investigate if a new approach of aerobic interval training (with light, moderate and high intensity periods) is detrimental/ineffective or beneficial from a biochemical point of view and, additionally, whether such findings correspond to the changes in the patient's quality of life. Our hypothesis is that such training method may improve quality of life and inflammatory profile, without elevate markers related to cell damage

Thus, the present study aimed to investigate the effects of 16 weeks of periodized AIT on a set of clinical biomarkers and quality of life in subjects with metabolic syndrome.

MATERIAL AND METHODS

Participants

Thirty six inactive untrained men and women (50.4 ± 6.7 years) diagnosed with metabolic syndrome (according to the international diabetes federation criteria)[1] were

included in the present study. Volunteers with chronic pulmonary diseases, neurological disorders, kidney failure and high-risk cardiovascular diseases (unstable angina pectoris, uncompensated heart failure, complex ventricular arrhythmias or myocardial infarction during the last month) were excluded from the study. In addition, participants with musculotendinous or osteoarticular injuries in the lower limbs and/or spine were also excluded. Such information was obtained through self-report.

The recruitment was conducted between 2015 and 2016 from the area around Presidente Prudente/SP, Brasil. Participants were recruited through publicity in media, such as television, internet and newspapers. Scientific events were also conducted to evaluate and prevent metabolic syndrome risk factors in the same city.

Oral and written information about the purpose and procedures of the study were provided, and a written consent was obtained from the participants. All procedures in the study were approved by the research Ethics Committee for studies involving human participants of the Universidade Estadual Paulista – UNESP, Presidente Prudente/SP (46509715.2.0000.5402), and were in accordance with the Declaration of Helsinki. The present study was registered in the clinical trials platform: *https://clinicaltrials.gov/ct2/show/NCT03036332.*

Aerobic Interval Training

The participants were allocated to AIT or control groups. AIT program consisted of three sessions per week for 16 weeks, between seven p.m. and nine p.m. However, the 6th, 11th and 14th weeks were designated as regenerative (without training). All exercise sessions were conducted on treadmill and supervised by physiotherapists with expertise on sport medicine. Each session started with 10 min of warm-up [general stretching plus

five minutes of walking at ≤ 19% of heart rate reserve (HRR)] and ended with a five minutes cool-down period.

Blood pressure assessment was performed between each effort and heart rate was continuously recorded throughout the training (by Polar Electro Oy Kempele, Finland – model S81Oi). In addition, participants in both groups were instructed to maintain their diet and daily activities during study period. In the AIT group, only subjects who performed at least 80% of the training sessions were included in the analyzes

Intensity

AIT was performed at three different workloads: five weeks of light intensities training on 20-39% of HRR; four weeks of moderate intensities between 40-59%HRR; and four weeks on high intensities of 60-90%HRR (table 1). At each stage, intensities at the upper limit were prioritized. However, when we previously performed a pilot study, with 70-90%HRR at high intensities period, some participants demonstrated raised blood pressure after efforts (systolic ≥200 mmHg and/or diastolic ≥110 mmHg) or musculoskeletal symptoms on lower limbs. Therefore, a greater range at this period (60-90%HRR) was needed to attenuate such occurrences.

Active recovery periods ranges from one to four minutes according to each volunteer, that is, a new effort would only start if the participant reaches the values: ≤19%HRR, ≤30%HRR and ≤50%HRR for the light, moderate and high workload periods, respectively. However, if such indexes were not reached after four minutes, a new effort began anyway. The speed of the treadmill was adjusted continuously to ensure the assigned heart rate in both exercise and recovery periods.

HRR was calculated according to the following description: HRR = (heart $rate_{max}$ – heart $rate_{rest}$) x training percentage load + heart $rate_{rest}$. The heart $rate_{max}$ was considered

as 220 – age in years, and for those patients with beta-blocker medication, a correction was perfomed to estimate heart rate$_{max}$[25].

Table 1. Aerobic interval training program.

Periods	Weeks	Sessions	Sets x bouts in minuts	Intensity % HRR
Light	1 - 2	1ª e 2ª	5 x 4	**Effort:** 20-39% HRR
		3ª e 4ª	6 x 4	
		5ª e 6ª	7 x 4	
	3	7ª, 8ª e 9ª	8 x 4	**Recovery:** ≤ 19% HRR
	4	10ª, 11ª e 12ª	9 x 4	
	5	**Regenerative**		
	6	13ª, 14ª e 15ª	9 x 4	
Moderate	7 - 8	16ª e 17ª	4 x 2,5	**Effort:** 40-59% HRR
		18ª e 19ª	5 x 2,5	
		20ª e 21ª	6 x 2,5	
	9	22ª, 23ª e 24ª	7 x 2,5	**Recovery** ≤ 30% da HRR
	10	25ª, 26ª e 27ª	7 x 2,5	
	11	**Regenerative**		
High	12 – 13	28ª e 29ª	5 x 1,5	**Effort:** 60-90% HRR
		30ª e 31ª	6 x 1,5	
		32ª e 33ª	7 x 1,5	
	14	**Regenerative**		**Recovery** ≤ 50% HRR
	15	34ª, 35ª e 36ª	8 x 1,5	
	16	37ª, 38ª e 39ª	9 x 1,5	

HRR: Heart rate reserve.

Bioelectrical Impedance

Bioelectrical impendence was measured using the octopolar TANITA®, model BC-418, segmental body composition analyzer, Iron Man/Inner Scaner. Participants were instructed to abstain from eating and drinking at least 4 hours prior; wear few clothes as possible, as well to refrain exercise for 24 hours before the analysis. The anthropometric characteristics were assessed using a stadiometer (Sany – American Medical do Brasil, São Paulo, Brazil) and checked by the same person to minimize interpersonal variation.

Blood Sampling

Blood samples was obtained by a trained nurse from an arm vein after overnight fasting. The participants was instructed to refrain any vigorous exercise the last 120hr prior blood collection, as well after the last training session. Everyday medication and water intake was allowed. Blood samples were immediately allocated into vacutainer tube containing EDTA and analyzed using standard procedures by a clinical analysis laboratory (UNILAB, Presidente Prudente-SP).

The serum concentration of CRP, creatine kinase (ck), creatine kinase - mb (ck-mb), creatinine, aspartate aminotransferase and alanine aminotransferase were assessed using kits Labtest and following the manufacturer's recommendations. High-sensitivity cardiac troponin T (cTnT), Hcy and complete blood count analyzes were assessed by high sensitivity electrochemiluminescence, chemiluminescence, and polarized multi angle dispersion methods, repectively.

Regarding the renal function, the estimated glomerular filtration rate (eGFR) was assessed according to the following formula: eGFR = 141 x $\min(S_{cr}/k, 1)^{\alpha}$ x $\max(S_{cr}/k, 1)^{-1.209}$ x 0.993^{age} x 1.018 [if female] x 1.159 [if black]. Abbreviations: eGFR= mL/min/1.73 m^2; S_{Cr} (standardized serum creatinine) = mg/dL; $\kappa = 0.7$ (females) or 0.9 (males); $\alpha = -0.329$ (females) or -0.411 (males); min = indicates the minimum of S_{Cr}/κ or 1; max = indicates the maximum of S_{Cr}/κ or 1; age = years[26].

Quality of life

Quality of life was measured in the baseline and after 16 weeks by the Medical Outcomes Study-Short Form 36 (SF-36), a questionnaire widely used and previously described in metabolic syndrome patients[27].

Statistical Analysis

Descriptive data were reported as mean value and standard deviation (SD). Analysis of covariance (ANCOVA) was performed to test differences between and intra group, using the mean difference (delta value) as dependent factor, group variable as fixed factor, and baseline values and sex as covariate. Analysis of covariance with bonferroni adjustment and levene´s test was used to assess homogeneity of data. The change in each group was reported as Estimated Marginal Means (EMM) and 95% Confidence Intervals (CI). The measures of effect size were calculated with ES-r: eta squared (0,01 small; 0,06 medium; 0,14 large)[28]. Creatine kinase data was transformed as base-10 logarithm to secure data homogeneity at Levene's test. In addition, eGFR data did not present homogeneity (even when transforming into base-10 logarithm) and a student's t test, paired and unpaired, was performed after Shapiro-wilk normality test.

P values <0.05 were considered significant and all statistical analysis were performed using SPSS (version 21.0).

RESULTS

Ninety-three subjects were allocated into one of the four groups, aerobic interval training, control group and two resistance training groups. The AIT and control groups had the same sample loss by approximately 32% (figure 1).

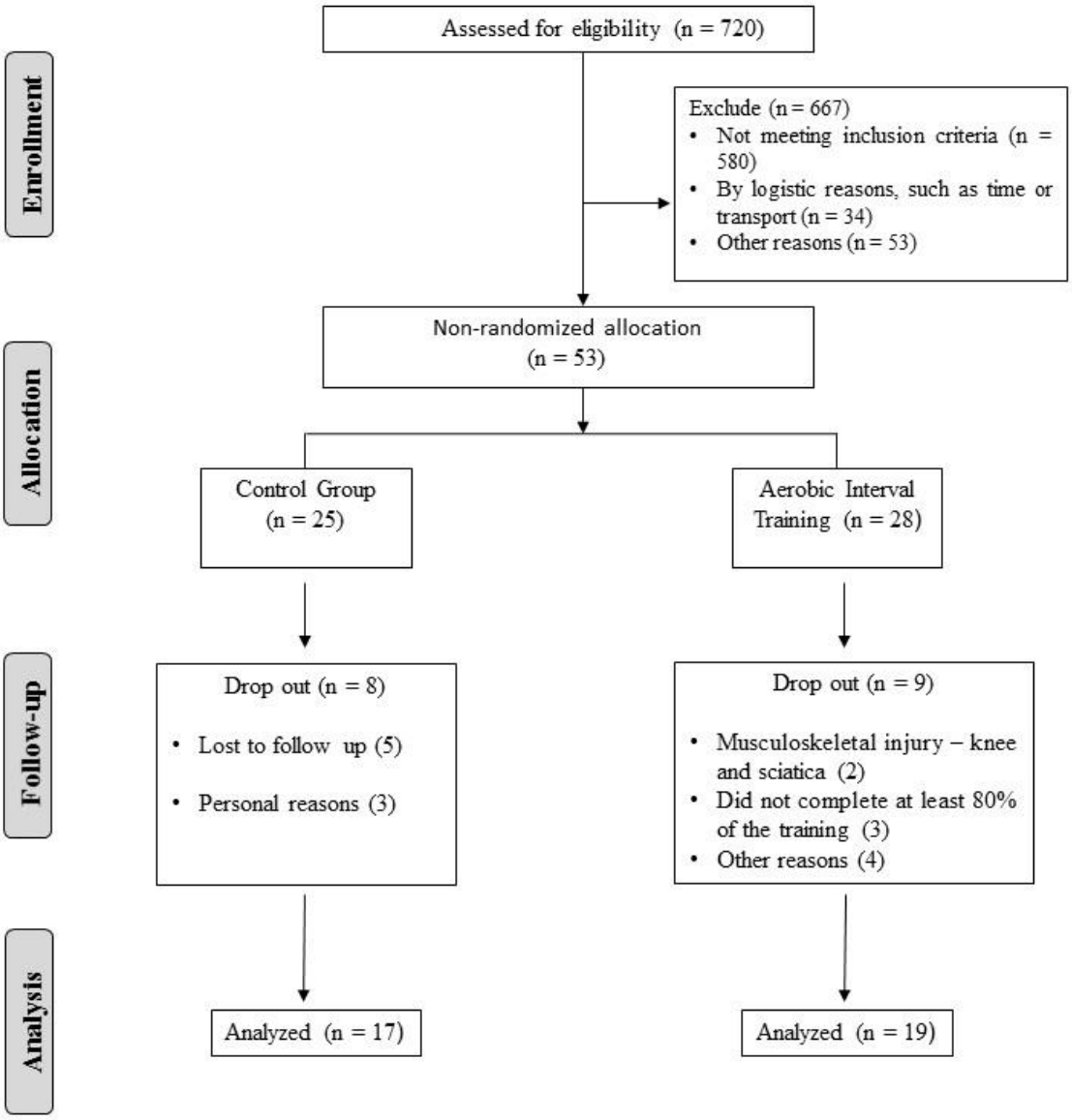

Figure 1. Flowchart of the participants.

Table 2 presents the participants characteristics, such as anthropometric values and daily medication at baseline in mean and SD. Significant differences were observed at baseline between groups for fat free mass and height values (p <0.05), but not for age, body mass index and fat mass (p >0.05)

Table 2. Patient characteristics (in mean and standard deviation) and medication use at baseline.

	Control group	Aerobic interval training	*p* value
Characteristics			
Age, years	52.59 ± 5.87	48.47 ± 6.95	0.065
Height, m	1.65 ± 0.07	1.72 ± 0.08	0.011*
Body mass index Kg/m^2)	29.99 ± 4.07	32.81	0.056
Fat mass %	34.95 ± 7.63	31.28 ± 6.98	0.141
Fat free mass (Kg)	53.15±10.47	67.01±11.55	0.001*
Sex (male – female)	(8 - 9)	(16 - 3)	
Medication use			
B-blockers	5	5	
Statins	4	4	
Metformin	5	1	
Ca^{2+} Antagonists	1	2	
Angiotensin II receptor blockers	7	6	
Diuretics drugs	3	6	
Insulin	2	1	
Others	5	5	

*Statistically significant difference between groups.

Biomarkers

Figures 2 and 3 show the frequency of biomarkers disorders in each group, according to the reference values used by the standard laboratory (see details at supplementary material - appendix 1). Surprisingly, both groups reduced the frequency of serum CRP, 25.9% to control group and 21.9% to AIT. However, only AIT was able to reduce the frequency values for high- sensitivity cardiac cTnT and the hepatic enzyme AST (figure 2).

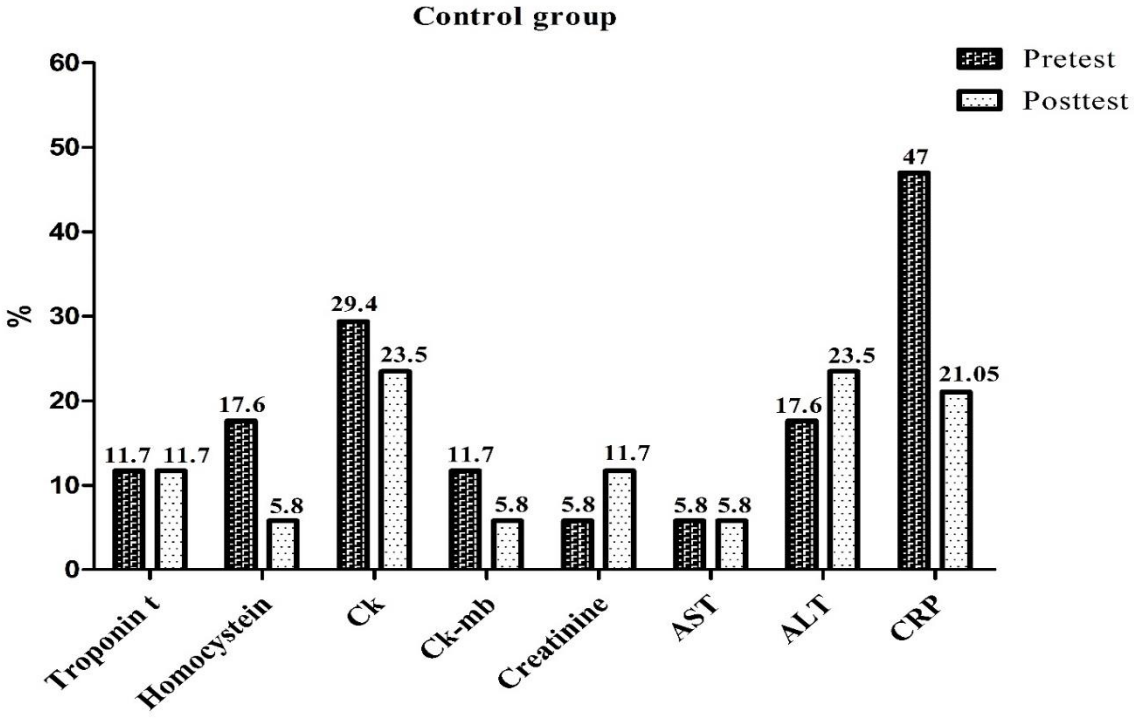

Figure 2. Occurrences of biomarkers disturbance at control group.

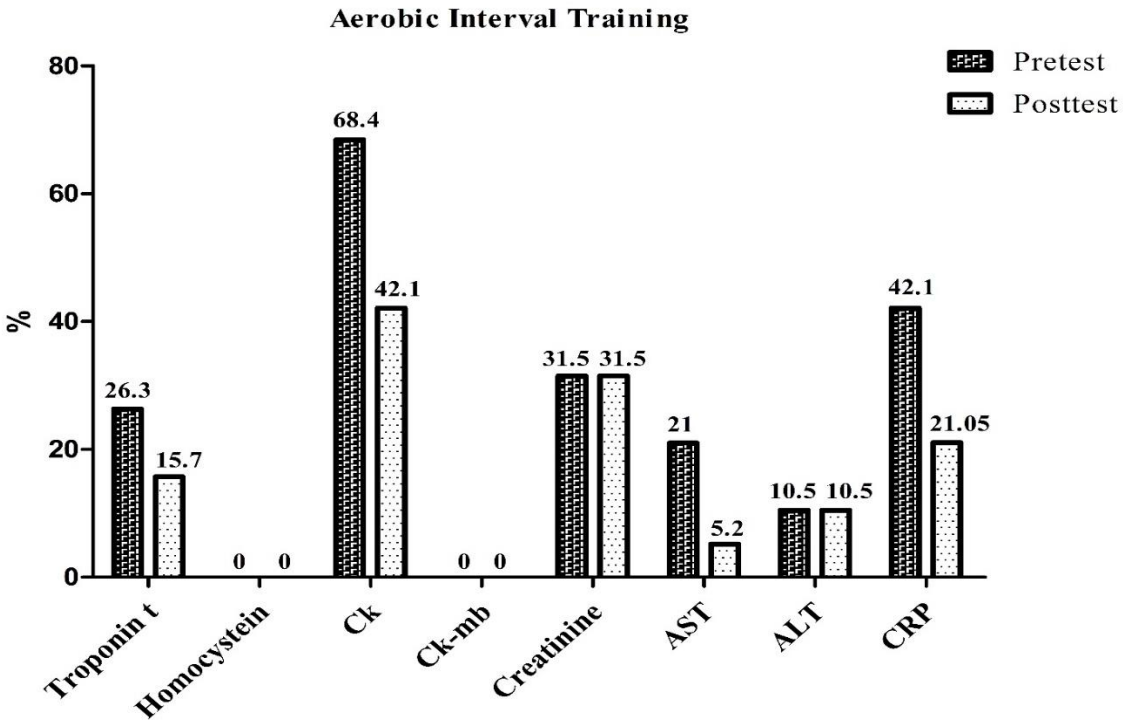

Figure 3. Occurrences of biomarkers disturbance at aerobic interval training.

Tables 3 and 4 shows the analysis of covariance adjusted by gender and baseline values for body composition and all biomarkers. Body weight was significantly increased in control group (EMM = 1.26; 95% CI 0.07; 2.45) and such change was significant compared to AIT (P = 0.03). No statistically significant changes were observed intra and between groups for the variables fat free mass, cTnT, ck-mb, AST, ALT, Hcy and hematological profile.

In the AIT group, serum CRP was significantly reduced by 36.3% between moments (from 6.0±4.0 to 3.82±3.43), whereas control group demonstrated a reduction by 22.3%, however, without significant difference. In addition, when we conducted an exploratory analysis without outliers (see details at supplementary files – appendix 2) there is a statistical difference between groups (P = 0.04) for CRP outcome with moderate effect size (table 5).

The estimated glomerular filtration rate did not change in both groups (AIT = from 76.73 ± 12.80 to 77.73 ± 13.48) and (control group = from 84.47±14.61 to 83.76±13.57), and no difference was observed between groups (P = 0.54).

Quality of life

In the table 6, our findings indicate that AIT significantly increased quality of life in most SF-36 domains (physical functioning = 3.4%; role limitations due to physical healt = 11.4%; vitality = 7.8%; and general health perceptions = 12.9%). In contrast, all domains remained significantly unchanged in control group, except for the role limitations due to physical healt (decreased by 5.7%). In addition, significant difference between groups was observed only in the domain Role limitations due to physical health with large effect size (P= 0.01, ES-r= 0.163).

Correlation

There was a significant correlation between change in body weight (kg) and change in CRP levels (Pearson correlation = 0.53, $P = 0.001$) and between change in Ck-mb and change in hepatic enzyme AST (Pearson correlation = 0.47, $P = 0.004$). Surprisingly, there was no significant correlation between change in fat mass and change in CRP (Pearson correlation = - 0.24, $P = 0.15$).

Table 3. Analysis of covariance for body composition and cardiac damage markers analysis expressed as estimated mean difference and interval confidence of 95%.

	Control group		Aerobic interval training		*p*	ES-r
	Pretest	Posttest	Pretest	Posttest		
Body composition						
Weight (kg)	81.99±15.10	83.21±15.13	97.83±16.48	97.23±16.88		
EMM	1.26		-0.64		0.03†	0.135
95% CI	(0.07; 2.45)*		(-1.75; 0.47)			
Fat free mass (kg)	53.15±10.47	53.61±10.13	67.01±11.55	66.39±12.63		
EMM	0.58		-.072		0.21	0.047
95% CI	(-0.83; 2.00)		(-2.05; 0.60)			
Cardiac damage markers						
cTnT (ng/mL)	0.0077±0.006	0.0078±0.007	0.0094±0.004	0.0090±0.004		
EMM	0.000		-0.001		0.37	0.025
95%CI	(-0.001; 0.002)		(-0.002; 0.001)			
CK (log)	2.08±0.27	1.99±0.28	2.36±0.29	2.22±0.25		
EMM	-0.13		-0.09		0.61	0.008
95% CI	(-0.24; -0.03)*		(-0.19; -0.001)*			
CK-mb (U/L)	14.31±7.80	14.51±7.59	14.38±4.89	12.45±4.92		
EMM	0.18		-1.92		0.25	0.040
95% CI	(-2.39; 2.77)		(-4,35; 0.51)			

Values are expressed as means ± SD, and results are expressed as estimated margins of the mean (EMM) and 95% confidence intervals (CI). ES-r: eta squared; cTnT: cardiac troponin T; CK: creatine kinase; CK-mb: creatine kinase mb. *Significant change from pretest to posttest; † Significant change between groups.

Table 4. Analysis of covariance for biomarkers analysis expressed as estimated mean difference and interval confidence of 95%.

	Control group		Aerobic interval training		*p*	ES-r
	Pretest	Posttest	Pretest	Posttest		
Liver damage markers						
AST (U/L)	26.50±7.92	25.68±8.92	30.91±8.20	26.77±7.05		
EMM	-2.23		-2.86		0.79	0.002
95% CI	(-5.57; 1.10)		(-6.00; 0.28)			
ALT (U/L)	30.14±15.89	31.80±14.63	34.10±11.76	30.97±11.96		
EMM	-0.66		-1.05		0.92	0.000
95% CI	(-6.76; 5.42)		(-6.79; 4.67)			
Inflammation and endothelial cell injury markers						
CRP (mg/L)	6.31±4.65	4.92±4.46	6.07±4.06	3.82±3.43		
EMM	-1.14		-2.47		0.23	0.044
95% CI	(-2.70; 0.41)		(-3.94; -1.01)*			
Hcy	11.04±3.75	10.20±4.45	13.00±9.16	12.79±9.30		
EMM	-1.04		-0.03		0.22	0.046
95% CI	(-2.20; 0.10)		(-1.12; 1.05)			
Hematological profile						
RBC ($\times 10^6 \cdot mm^{-3}$)	5.04±0.45	5.09±0.51	5.22±0.35	5.18±0.34		
EMM	0,10		0.21		0.72	0.004
95% CI	(-0.38; 0.58)		(-0.23; 0.66)			
Hgb (g/dL)	14.36±1.59	14.54±1.87	15.19±1.16	14.97±1.38		
EMM	0.35		-0.04		0.18	0.055
95% CI	(-0.06; 0.78)		(-0.44; 0.35)			
Hct (%)	42.61±3.82	43.22±4.38	44.82±3.29	44.30±3.59		
EMM	0.71		-0.60		0.09	0.087
95% CI	(-0.36; 1.78)		(-1.61; 0.40)			

Values are expressed as means ± SD, and results are expressed as estimated margins of the mean (EMM) and 95% confidence intervals (CI). ES-r: eta squared; AST: aspartate aminotransferase; ALT: alanine aminotransferase; CRP: C-reactive protein; Hcy: homocystein; RBC: red blood cells; Hgb: hemoglobin; Hct: hematocrit. *Significant change from pretest to posttest.

Table 5. Analysis of covariance for biomarkers analysis expressed as est*i*mated mean difference and interval confidence of 95% with out outliers.

	Control group		**Aerobic interval training**		***p***	**ES-r**
	Pretest	Posttest	Pretest	Posttest		
cTnT (ng/mL)	0.0056±0.001	0.0053±0.002	0.0094±0.004	0.0090±0.004		
EMM	0.000		0.000		0.96	0.000
95% CI	(-0.002; 0.001)		(-0.001; 0.001)			
CK-mb (U/L)	14.13±8.02	15.00±7.56	13.98±4.36	12.10±4.56		
EMM	0.72		-1.74		0.09	0.093
95% CI	(-1.32; 2.77)		(-3,72; 0.23)			
AST (U/L)	24.30±4.06	23.41±5.94	30.91±8.20	26.77±7.05		
EMM	-2.61		-2.77		0.94	0.000
95% CI	(-5.79; 0.56)		(-5.55; 0.002)			
ALT (U/L)	32.32±15.60	29.39±12.81	32.56±10.93	29.15±10.49		
EMM	-3.83		-2.61		0.69	0.005
95% CI	(-8.33; 0.66)		(-6.82; 1.59)			
CRP (mg/L)	6.31±4.65	4.92±4.46	5.92±3.90	3.36±2.79		
EMM	-1.01		-2.61		0.04†	0.125
95% CI	(-2.11; 0.53)		(-3.66; -1.55)*			

Values are expressed as means ± SD, and results are expressed as estimated margins of the mean (EMM) and 95% confidence intervals (CI). ES-r: eta squared; AST: aspartate aminotransferase; ALT: alanine aminotransferase; CK-mb: creatine kinase mb; cTnT: cardiac troponin t; CRP: C-reactive protein. *Significant change from pretest to posttest; † Significant change between groups.

Table 6. Analysis of covariance for quality of life analysis.

	Control group		Aerobic interval training		*p*	ES-r
	Pretest	Posttest	Pretest	Posttest		
Physical functioning	76.147±12.59	78.23±11.98	84.73±13,79	87,63±10,84		
EMM	-0.45		4.87		0.15	0.061
95% CI	(-5.61; 4.70)		(0.03; 9.72)*			
Role limitations due to physical health	77.94±31.72	73.52±33.62	80.26±29.55	89.47±15.17		
EMM	-5.58		10.26		0.01†	0.163
95% CI	(-14.61; -3.44)*		(1.75; 18.76)*			
Bodily pain	59.05±21.59	64.23±21.62	70.26±21.15	73.84±16.40		
EMM	2.89		5.62		0.61	0.008
95% CI	(-4.64; 10.42)		(-1.46; 12.70)			
General health perceptions	61.58±15.98	67.88±13.60	66.47±19.95	75.05±13.81		
EMM	4.93		9.79		0.26	0.038
95% CI	(-1.17; 11.05)		(4.03; 15.54)*			
Vitality	65.88±15.33	65.29±11.52	67.10±18.58	73.42±13.02		
EMM	-0.14		5.91		0.09	0.084
95% CI	(-5.15; 4.87)		(1.19; 10.63)*			
Social role functioning	72.05±27.78	73.52±25.34	83.55±16.16	87.50±18.16		
EMM	0.50		4.81		0.43	0.019
95% CI	(-7.11; 8.11)		(-2.33; 11.96)			
Emotional role functioning	74.51±36.38	72.55±39.50	77.18±40.14	73.67±34.39		
EMM	0.69		-5.89		0.59	0.009
95% CI	(-16.70; 18.09)		(-22.27; 10.48)			
Mental health	69.64±16.12	69,64±14.49	78.94±12.56	80.63±13.14		
EMM	-0.77		2.37		0.43	0.019
95% CI	(-6.37; 4.83)		(-2.89; 7.63)			

Values are expressed as means ± SD, and results are expressed as estimated margins of the mean (EMM) and 95% confidence intervals (CI). ES-r: eta squared. *Significant change from pretest to posttest; † Significant change between groups.

DISCUSSION

The aim of the present study was to investigate the effects of AIT on clinical biomarkers and the quality of life among metabolic syndrome. The main findings suggest that 16 weeks of a new approach of AIT on a treadmill (a) improve low-grade inflammation by reducing CRP concentration, (b) improve quality of life, and (c) did not present deleterious effects related to indirect cell damage markers. Therefore, such findings confirm our hypothesis that a periodized AIT, with an individualized recovery and workload control and progression, is effective in improving the quality of life and inflammatory profile without detrimental effects.

Anti-inflammatory response

Growing evidence suggests that obesity is a independent, but also modifiable, risk factor for several major diseases, including metabolic syndrome[29]. In the present study, 73.7% in the AIT group were classified as obese and 26.3% as overweight at baseline, whereas 41.2% were classified as obese and 58.8% as overweight in the control group. In this regard, hypertrophied adipose tissue can promote a cell hypoxia state[30, 31], which consequently augment local inflammatory cytokines by macrophages within adipose tissue and endothelial cells. In addition, such proinflammatory markers as interleukin 6 (IL-6) and TNF- α is considered the main pathway to induce CRP expression by the liver and trigger systemic inflammation[31].

In contrast, an anti-inflammatory response induced by exercise has been demonstrated, especially regarding to the acute effect of HIIT. Dorneles *et al* (2016)[32] performed a single bout of high-intensity interval exercise (10 × 60s of cycling efforts at 85–90% of maximal aerobic power, separated by 75-s at 50% of maximal aerobic power) among overweight-obese and observed enhancements of interleukin 10, an anti-inflammatory cytokine, by 27% (p=0.003). Similarly, Durrer *et al* observed a significant reduction of TNF-α at 1 hour post HIIT (7 X 60s, ~85% maximal aerobic power output, separated by 1-min recovery) among type 2 diabetes individuals[33].

However, such findings are not in agreement with those found after long-term intervention of HIIT. Several studies have not found significant changes for TNF, IL-6 and CRP concentration among metabolic syndrome, type 2 diabetes and overweight-obese subjects[4, 34-36]. Furthermore, Gerosa *et al*[37] have also observed a significant detrimental effect after 16 weeks of HIIT when compared to aerobic continuous training (30 min at 70% maximum heart rate) in metabolic syndrome. In such study, the authors indicate that HIIT group increased by approximately 104% (P=0.001) the concentration of TNF-α, whereas aerobic continuous training was effective to reduce such marker (P= 0.037).

Therefore, we believe that, although studies using HIIT programs have demonstrated benefits on cardiometabolic parameters and others[4,20,21], such protocols uses fixed intensities and established recovery periods, regardless of metabolic and systemic impairment, as occurs in metabolic syndrome. In this regard, the periodized training used in the present study, with light and moderate intensity periods, as well the individuality of recovery periods, was a key point to provide adaptation during each stage and minimize deleterious effects during higher intensities.

Cell damage biomarkers

Regarding cardiac and liver damage markers, there was no significant change intra and between groups, which suggests an abscence of detrimental effect from a molecular point of view (tables 3 and 4). cTnT is considered as a specific biomarker for myocardial injury and has been widely used to diagnose acute myocardial infarction[38]. In the AIT group, approximately 10% of the individuals reduced cTnT concentration (figure 3), which may suggests a more favorable condition after exercise

.However, studies have shown that acute exercise can increase serum concentrations of cardiac biomarkers, including cTnT. Ranjbar *et al*[39] compared the acute effects of HIIT versus

moderate-intensity continuous exercise in cTnT concentration among sedentary men. cTnT significantly increased immediately after both training groups and remained elevated at 1 hour post (p=0.05), however ck-mb, did not present any significant change after both groups. The authors suggested that such increase does not seem to be caused by the irreversible death of cardiomyocytes, and remained unknown.

Regarding ck response, both groups significantly reduced serum concentrations, where 26% of the AIT subjects improved such biomarker, compared to 5.8% of the control group (figures 2 and 3). However, we do not have an explanation for this large decrease, especially in the AIT group.

Quality of life

Although several studies have reported improvement of quality of life after HIIT, including chronic heart failure and coronary artery disease patients[40, 41], there is a gap in the literature regarding the behavior of quality of life in metabolic syndrome. Therefore, our findings highlights HIIT interventions as a tool to increase quality of life in metabolic syndrome. However, only domains related to the physical aspect were improved, while mental aspects remained unchanged. Such effect may have occurred probably due to the type of training session, in which the participants did not socialize with other volunteers during the training.

Limitations

The present study presents some limitations that must be considered. Significant variation of gender among the participants; non-randomized allocation in the groups; the non-use of treadmills with slope, which could have minimized mechanical stress in the lower limbs; and finally, the findings related to indirect cell damage should be taken with caution, since

several variables may interfere their concentration and were not controlled in the present study (alcohol use, high fat diet and others).

Therefore, larger randomized control trials are required to confirm our findings. Further research using such AIT approach combined to dietary control, as well the analysis of anti and proinflammatory cytokines, is needed to examine inflammatory response in this field.

CONCLUSION

The new approach of AIT improves chronic low-grade inflammation and quality of life in metabolic syndrome. In addition, such training program was safe and did not present detrimental effects related to cell damage markers. Our findings highlight periodized training as a key point of exercise prescription, and its use should be conducted to improve health status, especially among unhealth individuous.

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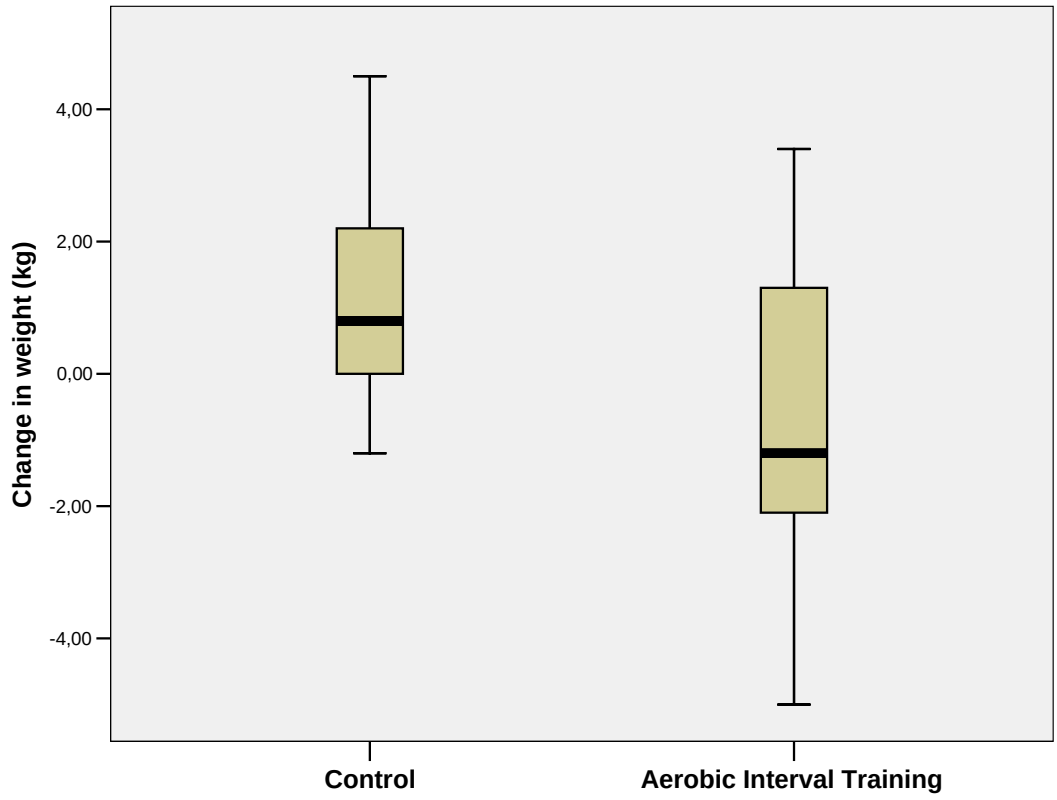
SUPPLEMENTARY FILES

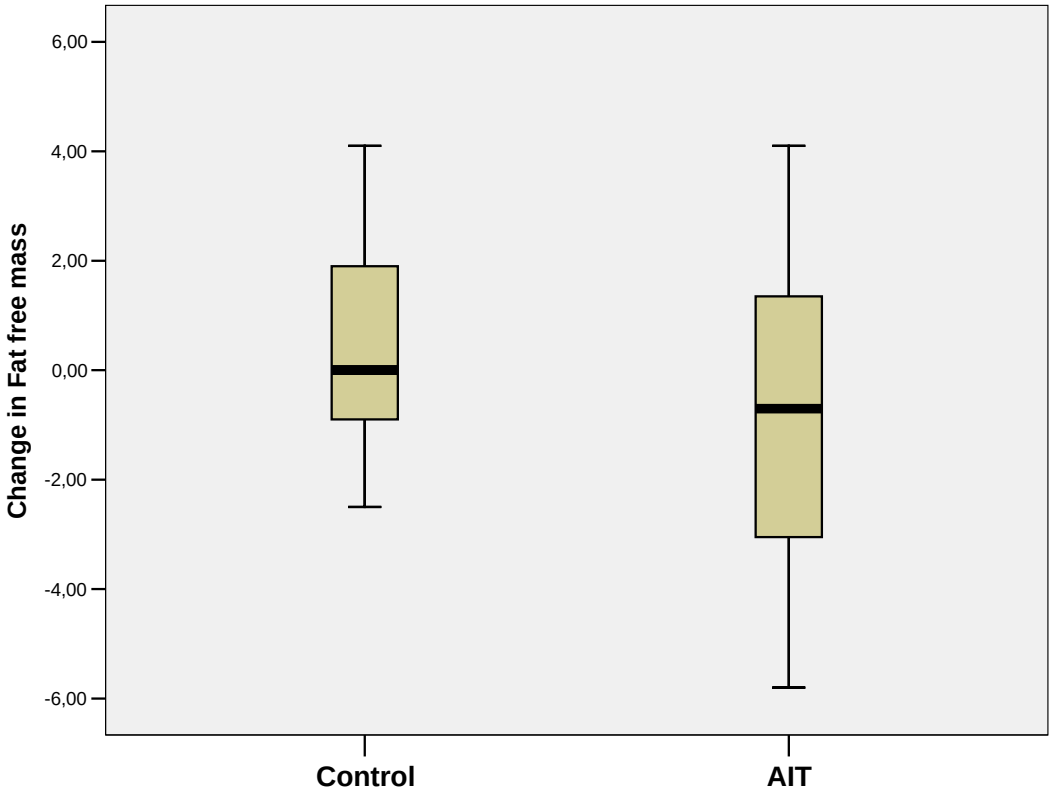
Appendix 1. Reference values used by the standard laboratory.

Biomarkers	**Men**	**Woman**
C-reactive protein - mg/L	< 6	< 6
Homocysteine - mcmol/L	5.46 to 16.20	4.44 to 13.56
Hs-cardiac troponin T – ng/mL	< 0.014	< 0.014
Creatine Kinase (mb) – U/L	< 25	< 25
Creatine Kinase – U/L	26 to 189	26 to 155
Aspartate aminotransferase – U/L	11 to 39	10 to 37
Alanine aminotransferase – U/L	11 to 45	10 to 37
Creatinine – mg/dL	0.70 to 1.20	0.53 to 1.0

U/L: units per liter; mg/L: miligram/liter; mcmol/L: micromol per liter; nanograms per milliliter. Hs: high-sensitivity

APPENDIX 2 – Median, minimum and maximal values, and outliers of each variable.

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-6,00
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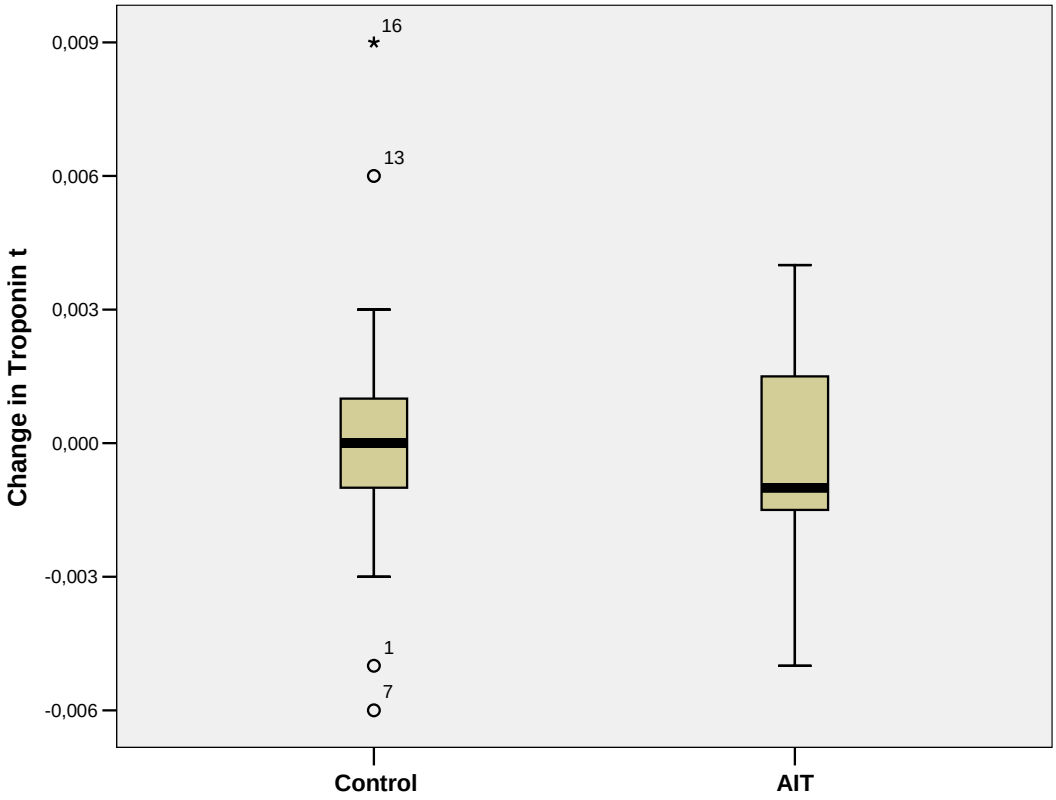
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1
7
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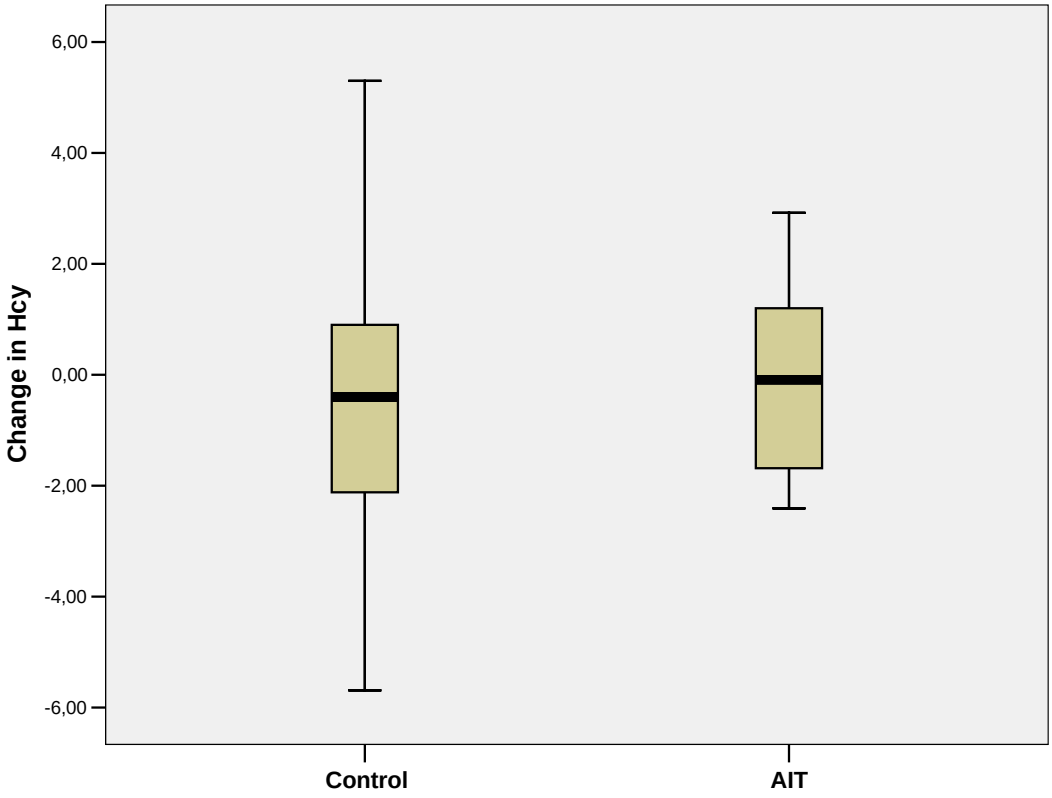
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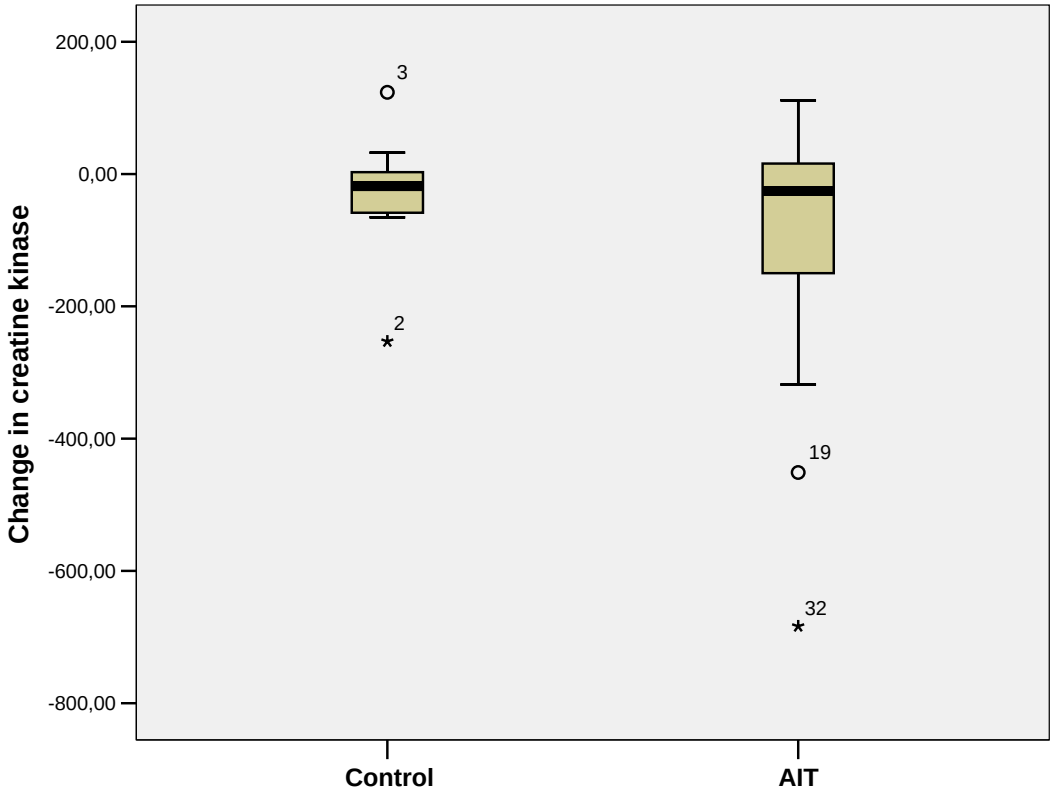
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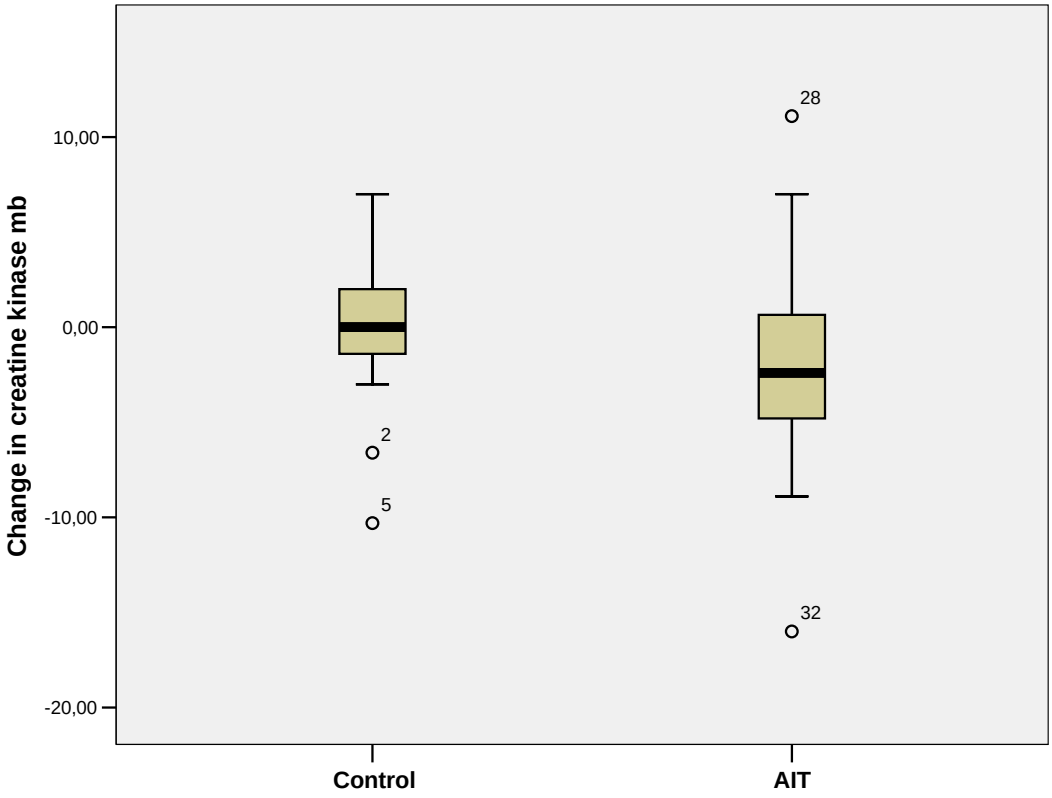
-800,00
3
2
19
32
Control
AIT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2
5
32
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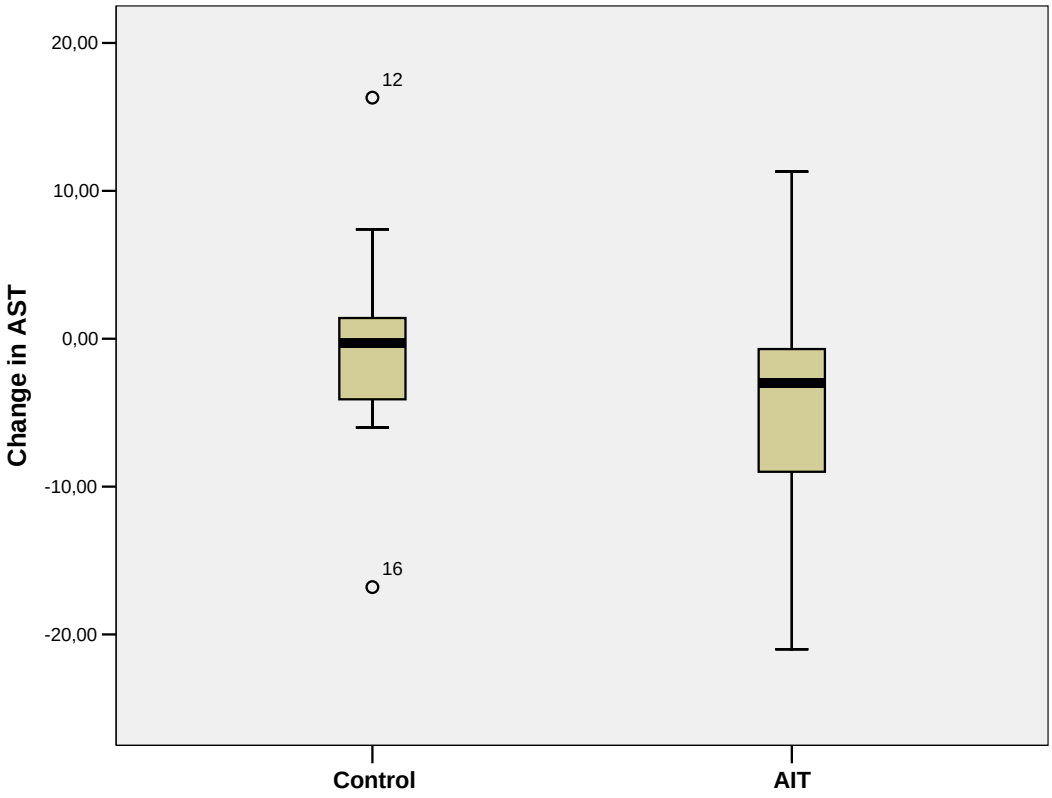
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16
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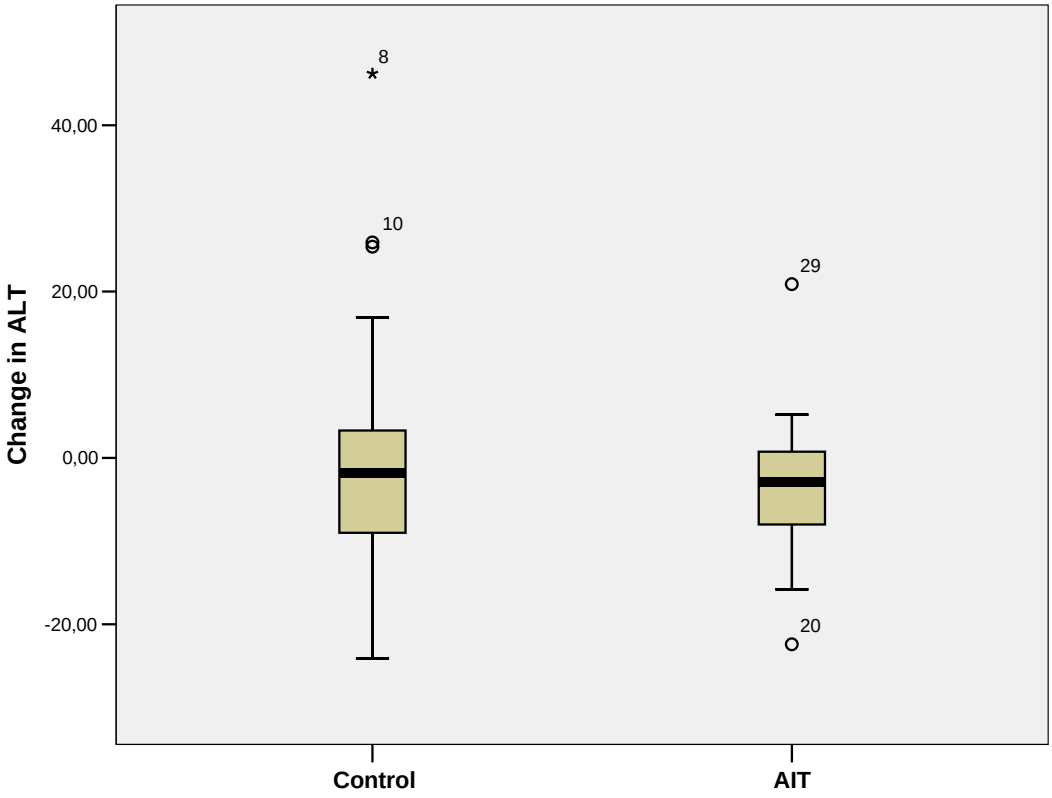
AIT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10
29
20
Control
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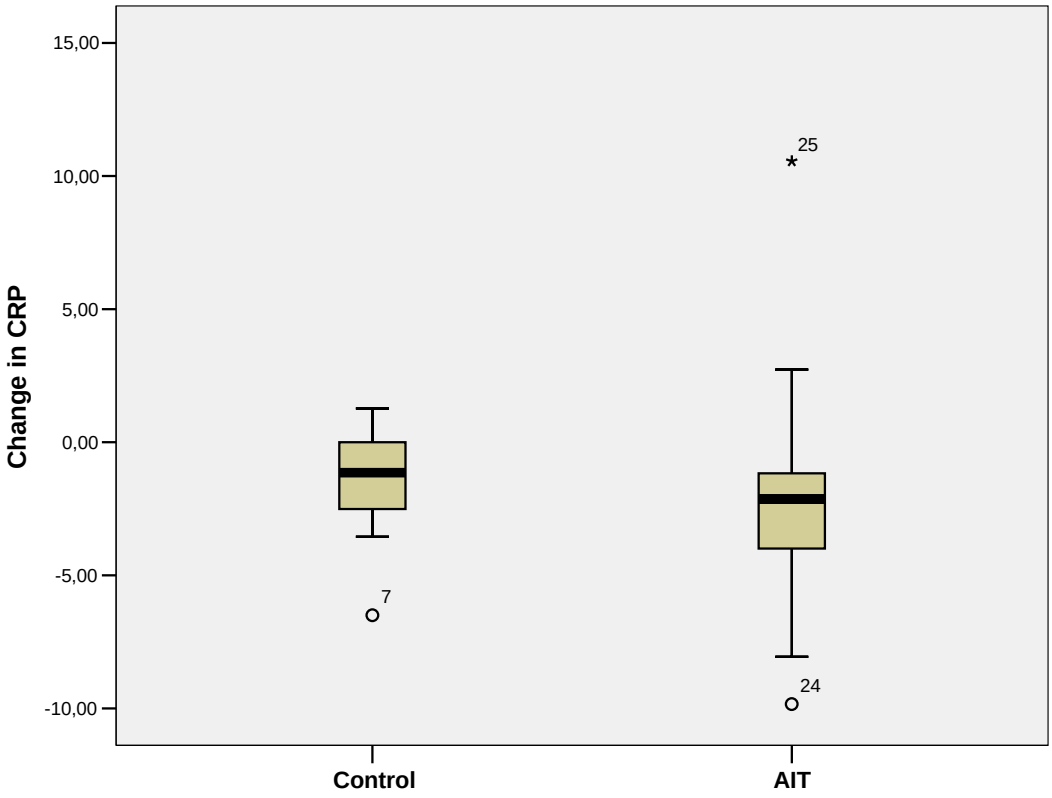
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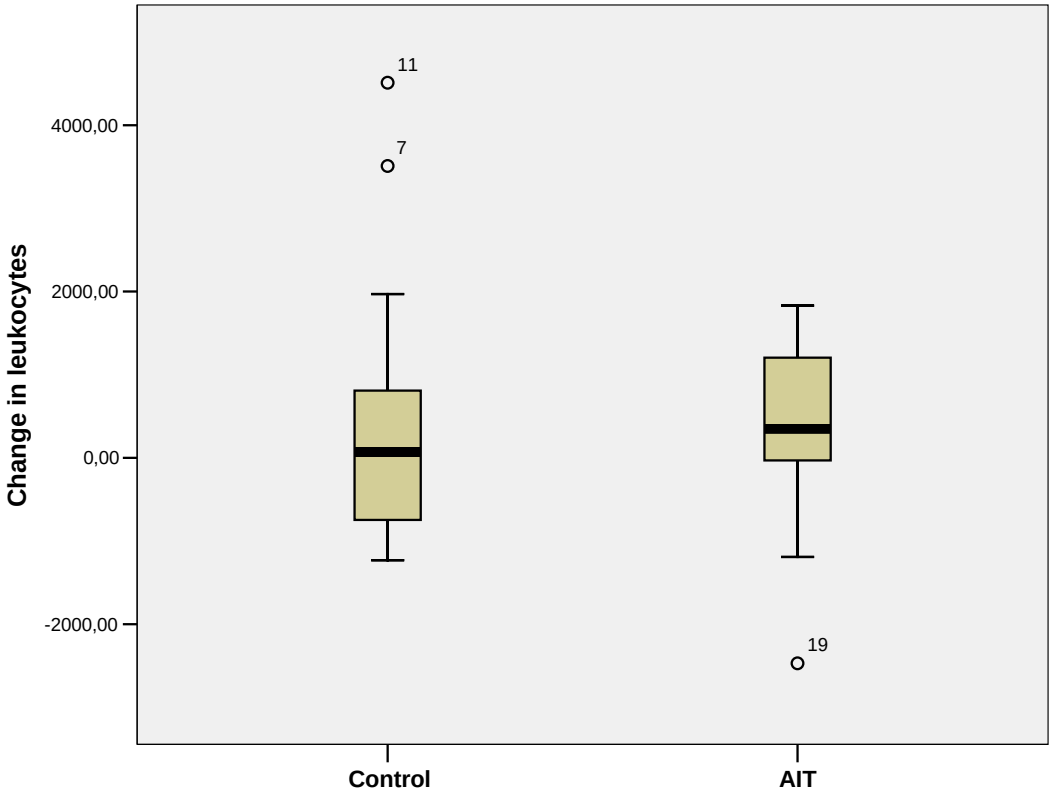
25
7
24
Control
AIT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11
7
19
Control
AIT

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Pesquisa: "EFEITOS DO TREINAMENTO PERIODIZADO AERÓBIO INTERVALADO SOBRE BIOMARCADORES CLÍNICOS, LIPOPEROXIDAÇÃO E QUALIDADE DE VIDA NA SÍNDROME METABÓLICA"

Nome do Pesquisador: Rodolfo Augusto Travagin Miranda.

Nome do Orientador: Jayme Netto Júnior.

1) *Natureza da pesquisa*: Você é convidado a participar desta pesquisa, que tem como objetivo analisar os efeitos que um programa de exercício aeróbio intervalado pode exercer sobre os biomarcadores clínicos (indicadores de saúde de diversos órgãos e sistemas corporais) e qualidade de vida em participantes com Síndrome Metabólica, ou seja, que tenham alterações nos seguintes aspectos: pressão arterial, glicemia, circunferência abdominal, triglicérides e colesterol.

2) *Participantes da pesquisa*: 40 participantes entre 35 e 60 anos e sedentários. Para fazer parte deste grupo você não pode fazer consumo de bebida alcoólica ou drogas. Além disso, você deverá assinar um termo em que declara ter passado por uma consulta médica e que encontra-se apto para realizar atividades físicas. Você permitirá que uma cópia deste atestado médico seja anexada a este termo.

3) *Envolvimento na pesquisa*: Ao participar deste estudo você deverá permitir que um exame físico e coleta sanguínea sejam realizados, além de um questionário avaliando as condições gerais de sua saúde. O sr (a) terá a oportunidade de obter um acompanhamento durante 16 semanas com a realização de exercícios em esteiras, ou, fazer parte do grupo controle onde serão realizadas apenas as avaliações iniciais e finais. Você terá que participar de todos os testes e das sessões de exercício seguindo o cronograma de horários, para que não haja comprometimento das análises das variáveis.

4) *Sobre o treinamento e coleta sanguínea*: O treinamento será realizado na FCT-UNESP, no CEAFIR (Centro de Estudos e Avaliações em Fisioterapia e Reabilitação), no setor de cardiologia, respeitando o horário das 19:00 às 21:00, com duração de aproximadamente de uma hora. A coleta sanguínea será realizada no Laboratório de Fisioterapia Desportiva (LAFIDE) por um profissional habilitado, onde, uma pequena amostra de sangue de 5ml será utilizada para análise dos biomarcadores sanguíneos. Os testes laboratoriais irão ocorrer no laboratório UNILAB, Centro de Análises Clínicas, na cidade de Presidente Prudente – SP.

5*) Protocolo experimental:* A semana que antecede o treinamento será destinada à realização de testes de familiarização na esteira, questionários de qualidade de vida e coletas sanguíneas. Seguidamente, caso o sr. (a) esteja no grupo treinamento, você participará de 16 semanas contendo 39 sessões de

exercício aeróbio intervalado, com uma frequência de 3 vezes por semana. E por fim, após o período de treinamento os procedimentos descritos acima serão realizados novamente.

6) *Riscos e desconforto*: Os procedimentos adotados nesta pesquisa obedecem aos Critérios da Ética em Pesquisa com Seres Humanos conforme Resolução no. 466/2012 do Conselho Nacional de Saúde. Deve-se destacar que você poderá sofrer micro-lesões nos músculos do seu corpo (lesões mínimas que são recuperadas rapidamente e de forma total), caracterizadas por dor muscular, como as que ocorrem normalmente após uma atividade intensa de exercícios, caracterizando uma situação comum e que não acarretará problemas a sua saúde. O monitoramento e a prescrição individualizada do treinamento minimizam quaisquer riscos de lesões graves ou intercorrências cardiovasculares durante o exercício. Caso o sr(a) apresente sensações como tontura, palidez, sudorese intensa, aumento excessivo da pressão arterial, dor ou qualquer outro sintoma, o exercício será interrompido imediatamente. Outro desconforto aparente poderá ser percebido durante as coletas de sangue.

7) C*onfidencialidade*: Todas as informações coletadas neste estudo são estritamente confidenciais. Seus dados serão identificados com um código, e não com seu nome. Apenas os membros da pesquisa terão conhecimento dos dados, assegurando assim sua privacidade.

8) *Benefícios*: Com a realização dos exercícios físicos propostos na presente pesquisa, o participante terá a possibilidade de melhoria nos parâmetros de qualidade de vida, bem como nos parâmetros bioquímicos analisados.

9) *Pagamento*: Você não terá qualquer tipo de despesa para participar da pesquisa e, nada será pago por sua participação.

A sra (sr.) tem liberdade de se recusar a participar e ainda se recusar a continuar participando em qualquer fase da pesquisa, sem qualquer prejuízo para a sra (sr.). Sempre que quiser poderá pedir mais informações sobre a pesquisa através do telefone do (a) pesquisador (a) do projeto e, se necessário através do telefone do Comitê de Ética em Pesquisa.

Após estes esclarecimentos, solicitamos o seu consentimento de forma livre para participar desta pesquisa. Portanto preencha, por favor, os itens que se seguem: Confiro que recebi cópia deste termo de consentimento, e autorizo a execução do trabalho de pesquisa e a divulgação dos dados obtidos neste estudo.
Obs: Não assine esse termo se ainda tiver dúvida a respeito.

Consentimento Livre e Esclarecido

Tendo em vista os itens acima apresentados, eu, de forma livre e esclarecida, manifesto meu consentimento em participar da presente pesquisa.

_____

Nome do Participante da Pesquisa

_____

Assinatura do Participante da Pesquisa

_____

Assinatura do Pesquisador

_____

Assinatura do Orientador

Artigo 2

Aerobic versus resistance training on inflammation status among patients with cardiometabolic disease: Systematic review and meta-analysis

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Abstract

Objective: To compare the effects of aerobic training (AT) *versus* resistance training (RT) on serum concentrations of C-reactive protein (CRP), tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6) among chronic diseases population. *Design:* Meta-analysis of randomized controlled trials (RCTs) comparing AT with RT. *Method*: Data were combined into the meta-analysis and described as standardized mean differences (SMD). Studies were selected after searching three databases (PubMed/MEDLINE, EMBASE and Cochrane Central Register of Controlled Trials - CENTRAL), from the earliest date of publication to the 7th of July, 2016. Only RCTs that compared AT versus RT on CRP concentration were included. The studies were required to have a population of chronic disease, recognisable by the common presence of cardiovascular risk factors (obesity/overweight, diabetes and metabolic syndrome). *Results:* 11 studies with 584 participants were included in the main meta-analysis. Similar anti-inflammatory response was observed between modes of exercise to decrease CRP (SMD = 0.45, 95% CI -0.05 to 0.95; $p = 0.08$) and IL-6 (SMD = 0.06, 95% CI -0.17 to 0.29; $p = 0.59$), whereas AT was significantly superior to RT on improving TNF-α concentrations (SMD = 0.62, 95% CI 0.13 to 1.11; $p = 0.01$). *Conclusions:* AT is superior to RT on decreasing proinflamatory TNF-α cytokine, whereas similar responses were observed to CRP and IL-6 outcomes.

Keywords: Exercise; Chronic diseases; Inflammation; Lifestyle: Obesity; Meta-Analysis.

1. Background

Lifestyle-induced chronic diseases can potentially alter cardiorespiratory fitness, muscle strength and quality of life[1, 2]. Chronic diseases such as obesity have high prevalence worldwide[3] and may also trigger metabolic disorders, altered blood pressure and insulin resistance, which increases the risk of premature death[4].

Besides it's function as energy storage, adipose tissue has been recognized as an endocrine organ due to the secretion of key cytokines from adipocytes, including proinflammatory tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6)[5, 6]. These cytokines are involved in the release of acute reactant C-reactive protein (CRP) from the liver and their concentrations may be altered in individuals with metabolic disorders.

Recognized as chronic low-grade inflammation, persist increase of CRP has been suggested as a predictor for cardiovascular disease[7, 8] and linked to the development of metabolic disorders, type 2 diabetes and neurodegenerative disease[9, 10]. However, chronic low-grade inflammation observed in illness has no apparent symptoms, which requires an assessment by biomarkers to evaluate inflammatory background.

On the other hand, appropriate lifestyle modifications, which include exercise, are considered essential for the treatment of chronic diseases[11]. Exercise has demonstrated anti-inflammatory responses among healthy and unhealthy subjects[12, 13], however, it remains unknown which training mode [aerobic training (AT), such as high-intensity interval training / moderate-intensity continuous training; or resistance training (RT)] is the most efficient to improve inflammation status. A previous meta-analysis[14] examined the effects of exercise on CRP and found a significant change after AT, compared to control group, but no alteration after RT or combined training. However, the study enrolled only patients with type 2 diabetes and there was no comparison between different modes of training.

Therefore, a systematic review comparing different modes of exercise would provide better knowledge about the anti-inflammatory responses of exercise and be useful to establish the optimal exercise prescription to improve chronic low-grade inflammation. Additionally, a direct comparison between training modalities may provide a more reliable approach regarding the therapeutic effect of

exercise. Thus, the present study aimed to summarize and compare the anti-inflammatory responses (CRP, TNF-α and IL-6) of AT versus RT among patients with lifestyle-induced chronic diseases, such as type 2 diabetes, hypertension, obesity and metabolic syndrome.

2. Methods

This systematic review was registered in the International Prospective Register of Systematic Reviews (PROSPERO; CRD42016048907) and it was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analysis[15].

2.1 Search Strategies

Studies were selected after searching three databases (PubMed/MEDLINE, EMBASE and Cochrane Central Register of Controlled Trials - CENTRAL), from the earliest date of publication to the 7th of July, 2016. The search strategy used a combination of the following Medical Subject Headings (MeSH) terms: randomized controlled trial, aerobic training, resistance training, inflammatory markers and chronic disease (see details supplementary material - appendix 1). No restrictions were applied to the language of the studies.

2.2 Studies Selection

Only randomized controlled trials (RCTs) that compared AT with RT were included. AT and RT were eligible for inclusion if they were supervised and had a minimum of eight weeks of duration. RCTs that used co-intervention as a diet intervention were also included if it was equal for all groups. Studies were eligible if the primary outcome CRP was assessed and if they included participants with cardiometabolic chronic diseases, recognisable by the common presence of cardiovascular risk factors (i.e. type 2 diabetes, hypertension, overweight/obesity and metabolic syndrome). In addition, such diseases have in common the poor lifestyle as the main contributor to their development.

The studies selection was conducted in three steps (title, followed by abstract and full text). It was performed by two independent researchers (RATM and AFM) and discussion was used to solve disagreements.

2.3 Data Extraction

Extracted data included final values of means, standard deviations (SD), sample size and was performed by two reviewers (RATM and AFM). When final values were not available, change scores were used. In addition, when SD values were not reported by researchers, these data were calculated using methods recommended by Cochrane Handbook for Systematic Reviews [23] or the authors were contacted.

This process was performed using a standardized form that included details, such as methodological characteristics. Disagreements between researches regarding data extraction were also solved by discussion.

2.4 Quality Assessment

All studies included were assessed according to methodological quality. Two reviewers (RATM and AFM) independently assessed the risk of bias using PEDro scale[16, 17]. If trials had already been assessed and listed on the PEDro database, such scores were adopted. Methodological quality was not inclusion criteria[18].

The Grading of Recommendations Assessment, Development and Evaluation (GRADE)[19, 20] approach was used to evaluate the overall quality of the evidence. Briefly, the overall quality was initially regarded as 'high' and downgraded by one level for each of these factors: risk of bias (more than 25% of participants from studies of low/moderate methodological quality – PEDro score <7 points); inconsistency of results (If ≤75% of participants from studies with findings in the same direction; imprecision (<300 participants in total for each outcome) and reporting bias (asymmetry of the funnel plot visually and by Egger test)[21]. If the Egger test[22] result was statistically significant (2-tailed P<0.100), we would downgrade the quality of evidence by one level, suggesting the presence of small study effect. Indirectness, a feature from GRADE, was not a relevant factor to this review.

2.5 Statistical Analysis

All meta-analysis calculations were conducted with the Review Manager - RevMan software (version 5.3, Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014). Data analysis was calculated using a random–effect model for CRP and TNF-α due to the high heterogeneity (I^2) of the studies, while a fixed-effect model was used for IL-6 analysis. Data were combined into the meta-analysis and described as Standardized Mean Difference (SMD) with 95% confidence intervals (95% CI). These data were calculated in order to compare the effect of different modes of exercise on inflammation status. In addition, subgroup analysis based on different intensities of AT (moderate versus high) and long term duration (>16 weeks) was also performed.

Treatment effect was calculated using SMD with 95%CI and interpreted as: 0.2 represents a small effect, 0.5 a moderate effect and 0.8 a large effect, suggested by Cochrane Handbook for systematic reviews of interventions[23].

3. Results

From 475 studies identified in the database search, 17 were chosen for full-text review. Of these, six RCTs were excluded. Figure 1 shows all the screening process based on PRISMA flow diagram.

Figure 1

Eleven RCTs comprised a total of 584 participants, ranging the years of publication from 2011[24] to 2015[25]. Six studies included an overweight or obese population[25-30]; four had investigated type 2 diabetes[13, 24, 31, 32] and only one included metabolic syndrome patients[33]. No studies were found including hypertensive patients.

Ten studies were conducted among adults and elderly adults, while only one was composed of adolescents[25]. In addition, few studies have been conducted only in men [26, 28, 29] and just one was performed among overweight/premenopausal women[30]. Exercise plus diet intervention were found in two studies [25, 30].

Regarding training period, the trials ranged from three to nine months and the exercise frequency from three to four times per week. AT was performed on treadmill[26, 30, 32, 33], cycle ergometer or cross

trainer[24, 27, 29, 31], nordic walking[28], or any of the following interventions by preference (treadmill, cycle ergometer or cross trainer)[13, 25]. For RT intervention, the exercise prescription was based on one maximum repetition in most studies, and isokinetic training was not used (see details in table 1).

3.1 Aerobic versus Resistance Exercise on Inflammatory Markers

A general analysis of the effects of exercise training on CRP concentration included 11 studies[13, 24-33]. Figure 2 shows no significant pooled effect between AT and RT (SMD = 0.45, 95% CI -0.05, 0.95; $p = 0.08$).

Seven studies[24, 26-30, 33] were included in the analysis for pro-inflammatory cytokine TNF-α. The pooled results shows that AT achieved greater effectiveness than RT to decrease TNF-α concentration (Figure 3; SMD = 0.62, 95% CI 0.13, 1.11; $p = 0.01$) with effect size considered as moderate (SMD>0.5). In addition, when excluded the only study with statistical difference between groups[24], the superior effect of AT still remains to TNF-α (SMD = 0.25, 95% CI 0.01, 0.48; p = 0.04) and shows lower heterogeneity across studies ($I^2 = 19\%$).

The same seven studies were included in the analysis for IL-6 cytokine[24, 26-30, 33]. The analysis showed similar anti-inflammatory response across exercise modes (Figure 4; SMD = 0.06, 95% CI -0.17, 0.29; $p = 0.59$).

Figure 2

Figure 3

Figure 4

3.2 Secondary Exploratory Analysis

Studies[13, 24, 27-30, 32] using moderate-intensity aerobic exercise (i.e. ≤80% MHR, HRR or $Vo2_{max}$) were more effective than RT with a significant pooled effect (figure 5; SMD = 0.79, 95% CI 0.03, 1.55; p = 0.04) to reduce CRP. The effect size for this analysis was considered as moderate (SMD>0.5).

Similarly, studies that have performed exercise intervention for more than 16 weeks[13, 25, 27, 32] also showed significant superiority from AT on CRP (figure 6; SMD = 1.51, 95% CI 0.17, 2.85; p = 0.03). The effect size was considered as large (SMD>0.8).

Figure 5

Figure 6

3.3 Methodological quality of included studies.

The mean PEDro score from included studies was 4.8/10. Due to the nature of the interventions, blinded samples and therapists were not possible. Only two studies[13, 32] performed assessor blinding and only one implemented intention to treat analysis[25]. In addition, few studies reported concealed allocation[25, 33]. Complete details regarding risk of bias are reported in see details in electronic supplementary material - appendix 2.

Regarding the overall quality of evidence, measured by GRADE, this sistematic review provides very low quality to the outcome CRP, low quality to IL-6, and moderate quality to TNF-alpha, which can be observed on table 2. The inspection of funnel plot was conducted just for the CRP, since this variable have more than ten studies. The inspection of the funnel plot (figure 7) and the statistical significance on Egger test (P = 0.044) suggest serious small study effect for CRP, which also downgraded the quality of evidence by one level.

Figure 7

4. Discussion

This systematic review aimed to summarize and compare the effects of AT and RT on inflammation status among lifestyle-induced chronic diseases. The main finding suggests that AT is significantly superior to RT on improving TNF-α cytokine, whereas there was no difference between modes of exercise among CRP and IL-6 variables.

Recently, in an attempt to explore exercise benefits, studies have investigated anti-inflammatory responses from different modes of exercise and specific inflammatory cytokines analysis[12, 13, 25, 38]. However, different molecular responses are expected from different training stimuli[36]. According to the training specificity, AT recruits large muscle groups with a systemic energy demand, while RT works briefly against a resistive load of single muscle groups, with a local feature.

In the present study, it seems that AT promoted greater energy expenditure, since the studies did not report similar caloric expenditure between training modalities. However, higher energy expenditure plays a central role on anti-inflammatory response[37]. Physiologically, cortisol and serum IL-6 are increased in a training session[38] and provide metabolic modulations to keep muscle activity, such as lipid oxidation in the skeletal muscle as well enhances anti-inflammatory cytokines (IL-10)[39].

Additionally, IL-10 is capable to inhibit nuclear transcription factor Kappa B (NF-kB), the main transcription factor of TNF-α[37, 40], which may explain the improvement of TNF-α after exercise, especially after AT. However, studies have also suggested an anti-inflammatory effect by augmenting soluble TNF-α receptor, an outcome not assessed in this review, but capable to inhibit TNF-α cytokine[41, 42].

Regarding CRP biomarker, several studies have examined the effects of aerobic and resistance training to improve such outcome, an independent predictor for cardiovascular diseases[7, 8]. Although a previous meta-analysis[14] demonstrated that AT, but not RT or combined training, was effective on improving CRP concentrations (compared to control group), the present study highlights that AT with long-term interventions (>16 weeks) and moderate intensity (≤80% MHR, HRR or $Vo2_{max}$) may be required to reach better outcome versus RT.

In contrast, similar anti-inflammatory responses was observed after AT with moderate and high intensities. Cabral *et al*[12] compared two experimental sessions among physically active men [high-intensity intermittent exercise (1:1 at 100% at $vVO2_{max}$) versus moderate-intensity continuous exercise (70% of $vVO2_{max}$) with matched volume (5km)], and both exercise groups exhibited an increase of IL-10 and the IL-10/TNF-α ratio. However, further studies are still required to establish the sufficient and optimal training load (intensity and volume) to provide an effective anti-inflammatory response.

To the authors' knowledge, this is the first systematic review and meta-analysis to compare the effects of different modes of exercise on inflammation status. The strengths of the present study is its search protocol, which includes only RCTs and studies published in any language. Another strenght is the moderate quality of the evidence for TNF-α, which may be considered for clinical recommendations[23]. From a practical point of view our findings regarding TNF-α is interesting for public health, since the persist augment of such cytokine has been associated to insulin resistance and type II diabetes[43, 44].

As limitation of the study, high heterogeneity across studies was observed for the outcome CRP and subgroup analysis; second, all included studies exhibited low/moderate methodological quality (PEDro score<7pontos); and third, publication bias has been detected in the main meta-analysis (CRP), which suggest serious small study effect.

5. Conclusion

Although the practice of regular exercise is the key message to improve such chronic conditions, our findings suggest that AT provides greater anti-inflammatory response than RT on decreasing TNF-α cytokine, whereas similar responses was observed to CRP and IL-6. Further studies with better methodological quality and similar caloric expenditure between training modalities would provide better knowledge about exercise and chronic low-grade inflammation.

Practical implications

- Our findings suggest that aerobic training should be used primarily to reduce pro-inflammatory cytokine TNF- α, a signaling protein involved in systemic inflammation.
- From a practical point of view, the superiority of aerobic training over TNF-α is interesting for public health, since the alteration of such biomarker has been associated to insulin resistance and type II diabetes.
- The overall quality of the evidence in this systematic review ranged from very low to moderate, which highlights the need for higher-quality studies.

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Table 1. Characteristics of the included studies.

Study	Population / intervention	Training programs	Outcomes and time points analysed	PEDro
Alberga *et al* 2015[25]	**Obese adolescents** **n = 105**	22 weeks, 4 days per week	Hs-CRP after training	6
	Dietary counselling + AT = 55 Age = 15.5 ± 1.4	Treadmill, cycle ergometer or elliptical machine at 70% – 85% MHR.		
	Dietary counselling + RT = 50 Age = 15.9 ± 1.5	2 to 3 sets of 6–15 repetitions at the maximum load possible.		
Nikseresht *et al.*, 2014[26]	**Obese men n = 22**	12 weeks, 3 days per week	CRP IL-6 TNF-α After training	4
	AIT = 10 Age = 39.6 ± 3.7	Treadmill: 4 x 4 min at 80 – 90% of MHR, with active recovery of 3-minute jogging at 55–65%.		
	Nonlinear RT = 12 Age = 40.4 ± 5.2	40–65 minutes of weight training; ~ 10 exercises between 40% - 95% 1RM.		
Kohen *et al.*, 2013[27]	**Overweight n = 44**	22 weeks, 3 times/week	CRP IL-6 TNF-α After training	4
	AT = 25 Age = 35.69 ± 8.07	Cycling or cross trainer: 50 - 60% HRR.		
	RT = 19 Age = 36.46 ± 8.9	Circuit training - 8 exercises at 50 - 60% of the 15RM.		
Kadoglou *et al.*, 2013[13]	**Type 2 diabetes** **n = 44**	6 months, four sessions/week	Hs-CRP	6

	AT = 21 Age = 58.3 ± 5.4	Treadmill, cycling or calisthenics; 45 minutes at 60 – 75% of MHR.	After training	
	RT = 23 Age = 56.1 ± 5.3	2–3 sets (8–10 repetitions/exercise) of eight types of exercises at 60–80% of 1-RM.		
Venojärvi *et al.*, 2013[28]	**Overweight/obese men n = 75**	12 weeks, three times per week for 60 min	Hs-CRP IL-6 TNF- α	4
	AT = 39 Age = 55 ± 6.2	Nordic walking - 55% to 75% of HRR.		
	RT = 36 Age = 54 ± 6.1	50% to 85% of five-repetition maximum (5RM).	After training	
Donges *et al.*, 2013[29]	**Overweight men n = 26**	12 weeks, 3 days per week	CRP IL-6	5
	AT = 13 Age = 45.4 ± 6.12	Cycle ergometer with elliptical cross training at 75% – 80% of MHR.	TNF-α	
	RT = 13 Age = 51.7 ± 7.56	9 exercises at 75% - 80% 1RM	After training	
Stensvold *et al.*,2012[33]	**Metabolic Syndrome n = 21**	12 weeks, 3 days per week	CRP IL-6	6
	AIT = 11 Age = 49.9 ± 10.1	Treadmill: 4 x 4 min at 90% of MHR, with active recovery at 70% of peak heart rate.	IL-18 TNF-α	
	RT = 10 Age = 50.9 ± 7.6	Weight training at 60% - 80% 1RM. Time session between 40' and 50'.	After training.	
Swift *et al.*,2012[32]	**Type 2 diabetes** **n = 108**	9 months, 150 minutes per week	CRP	5
	AT = 58 Age = 55.8 ± 7.9	Treadmill - 50% to 80% of maximal oxygen consumption with 12 kcal/kg/week.		
	RT = 50 Age = 58.7 ± 8	9 exercises with 2 – 3 sets (10–12 repetitions/exercise). No reported intensity.	After training	

Sukala *et al.*, 2012[31]	**Type 2 diabetes n = 18**	16 weeks, 3 days per week	CRP	4
	AT = 9 Age = 51 ± 4	Cycle ergometer: 65% to 85% of HRR.		
	RT = 9 Age = 48 ± 6	2 – 3 sets (6-8 repetitions/exercise) to neural fadigue.	After training	
Fisher *et al.*, 2011[30]	**Overweight and premenopausal women n = 97**	Three times per week until a BMI <25	CRP TNF-α	4
	AT + Diet = 43 Age = 20 to 41 years	Walking/running on treadmill at 65% to 80% of MHR.	IL-6	
	RT + Diet = 54 Age = 20 to 41 years	10 exercises with 1 - 2 sets of 10 repetitions at 60% to 80% RM.	After weight reduced	
Jorge *et al.*, 2011[24]	**Type 2 diabetes n = 24**	12 weeks, 3 days per week for 60 min	Hs-CRP TNF-α	5
	AT = 12 Age = 52.09 ± 8.71	Cycling at the heart rate corresponding to the lactate threshold.	IL-6	
	RT = 12 Age = 54.10 ± 8.94	Consisted of a 7-exercise circuit.	After training	

Age: years (mean ± SD); **AT:** Aerobic Training; **RT:** Resistance training; **AIT:** Aerobic interval training; **Hs-CRP:** High-sensitivity C-reactive protein; **CRP:** C-reactive Protein; **IL-6:** Interleukin-6; **TNF-α:** Tumor necrosis factor; **HRR:** Heart rate reserve; **MHR:** Maximum heart rate.

Table 2. The overall quality of evidence (GRADE).

Outcomes	Quality assessment				Number of Patients		Effect	Quality
	Risk of bias[1]	Inconsistent[2]	Imprecision[3]	Reporting Bias	Resistance Training	Aerobic Training	Std. Mean Difference (95% IC)	
CRP	Limitation	Limitation	No limitation	Detected *	288	296	0.45 [-0.05, 0.95]	Very Low + + - +
TNF-alpha	Limitation	No limitation	No limitation	#	156	153	0.62 [0.13, 1.11]	Moderate + - - -
IL-6	Limitation	Limitation	No limitation	#	156	153	0.06 [-0.16, 0.29]	Low + + - -

[1] More than 25% of participants from studies of low or moderate methodological quality (PEDro score <7pontos); [2] If ≤75% of participants from studies with findings in the same direction; [3] Fewer than 300 participants for each outcome. # Was not performed due to the insufficient number of studies (<10 studies). * Inspection of funnel plot asymmetry and the Egger test were significant (P = 0.044).

1

FIGURES AND LEGENDS

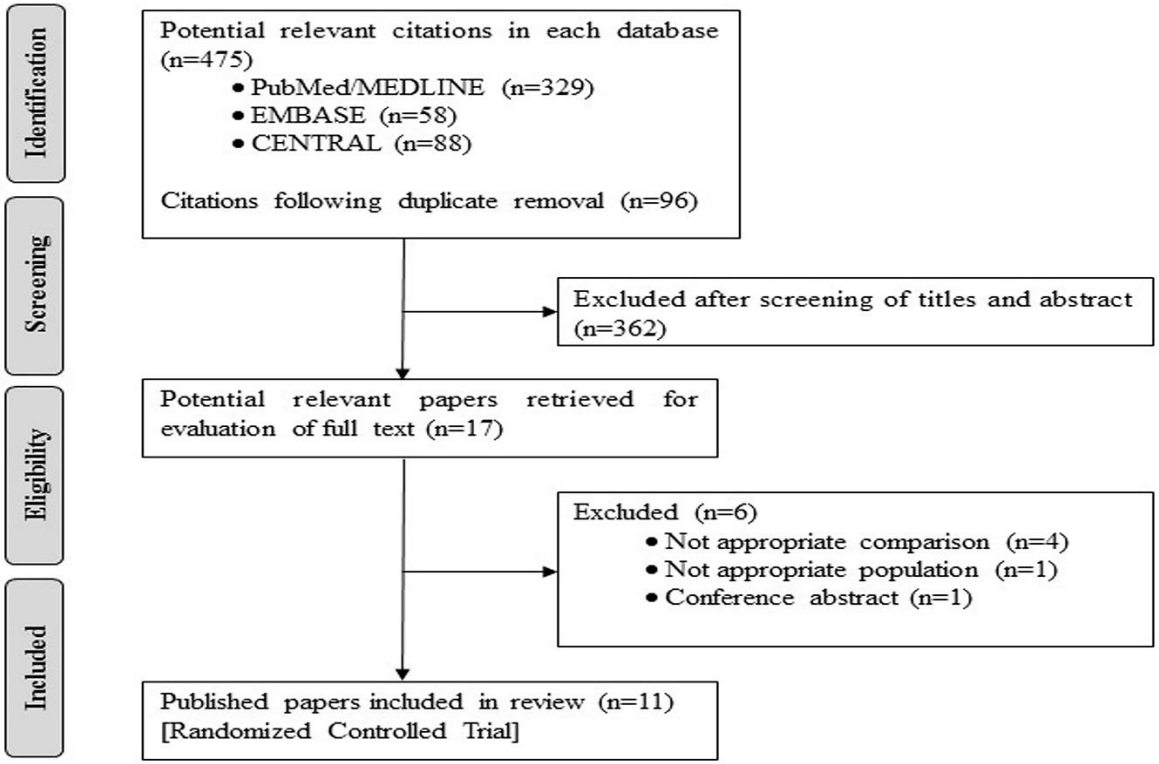

Figure 1. Flowchart of included studies.

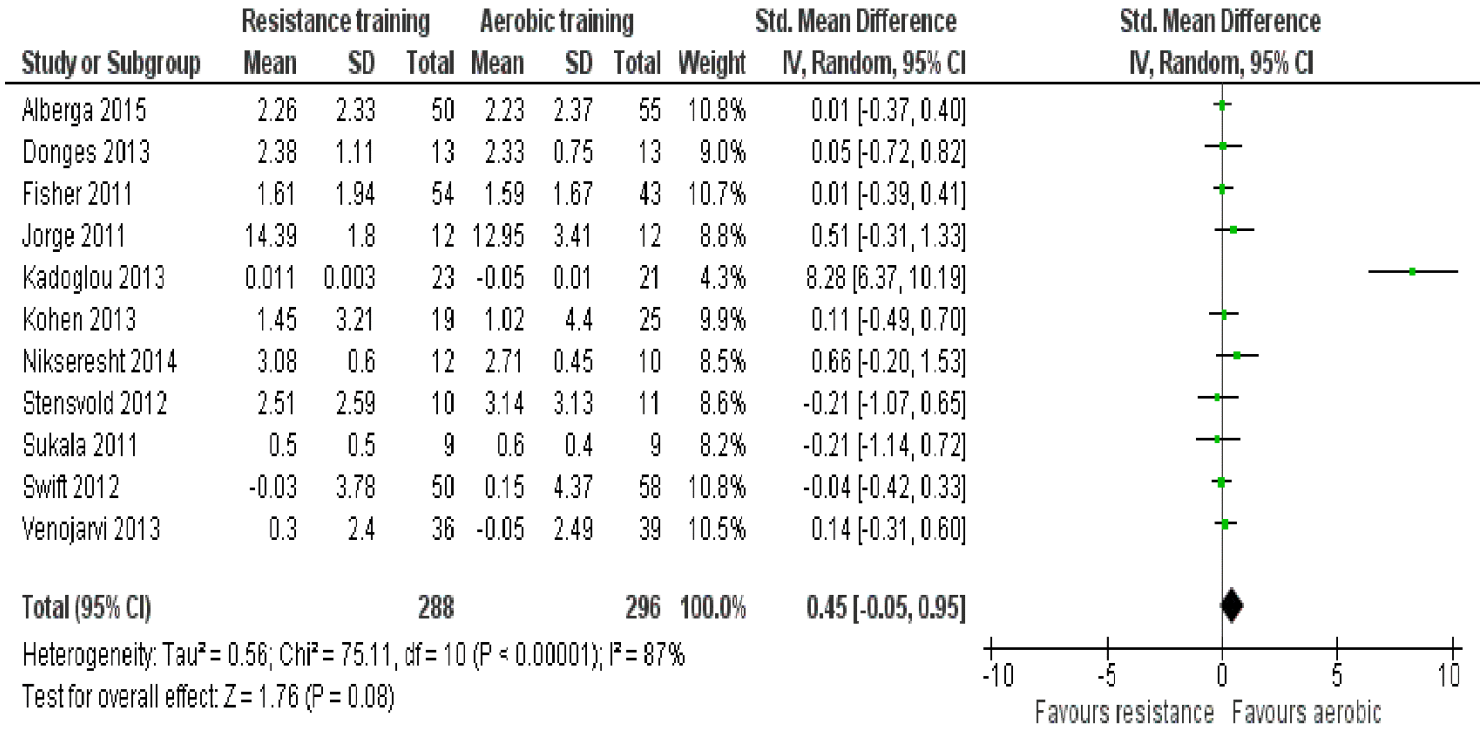

Figure 2 – Florest plot: effects of aerobic *versus* resistance training on C-reactive Protein.

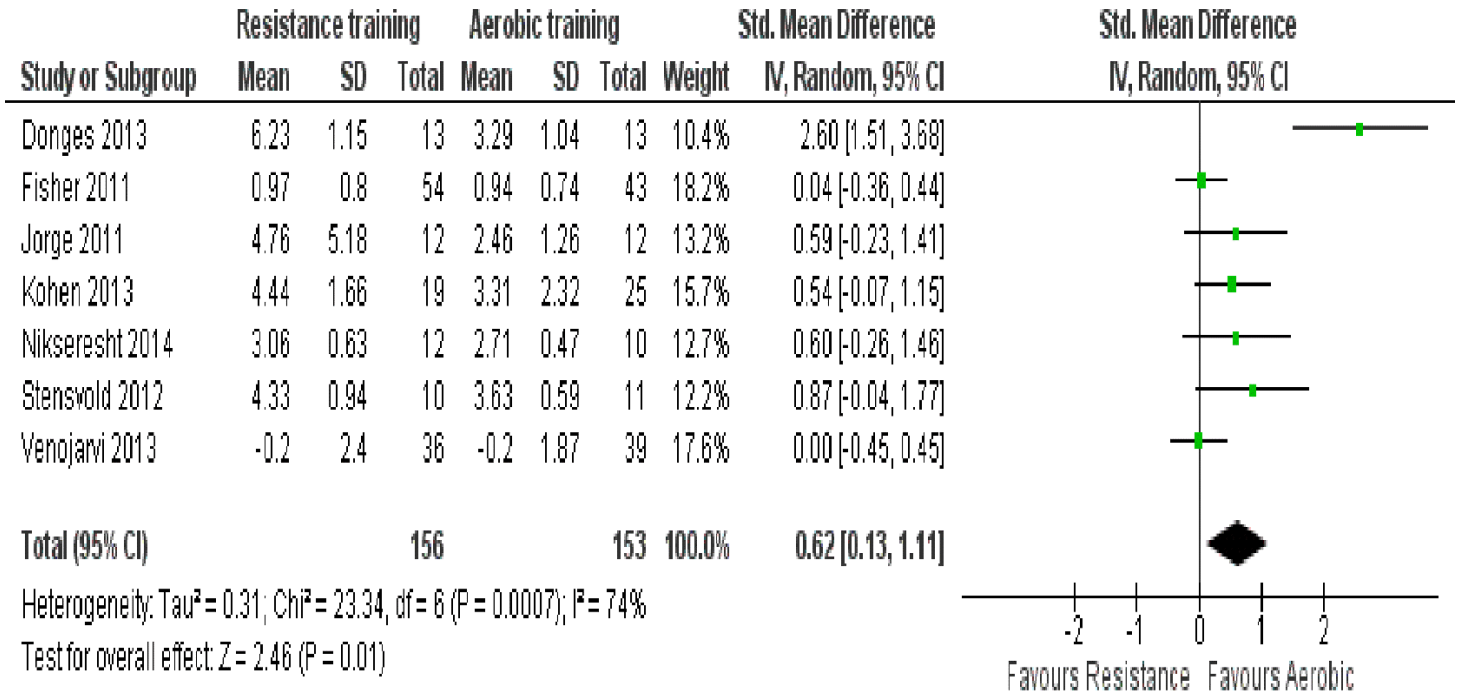

Figure 3. Florest plot: effects of aerobic *versus* resistance training on TNF-α concentration.

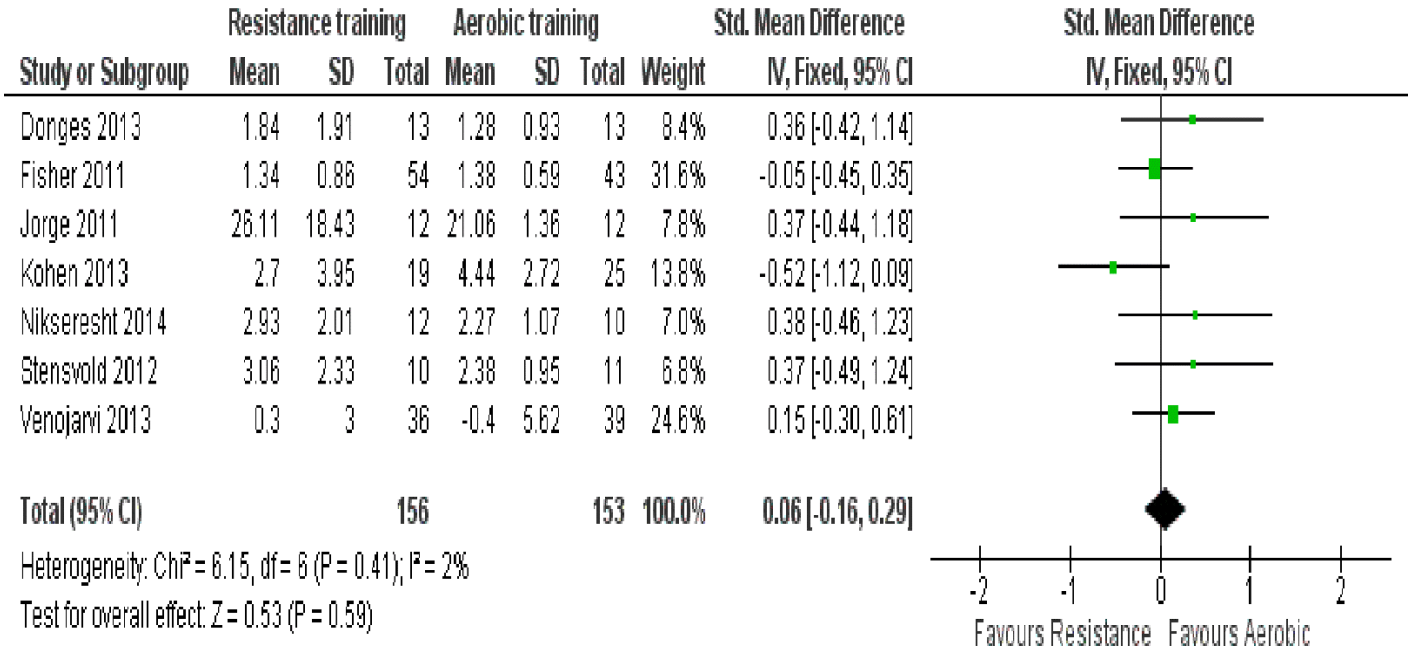

Figure 4. Flores plot: effects of aerobic *versus* resistance training on Interleukin-6.

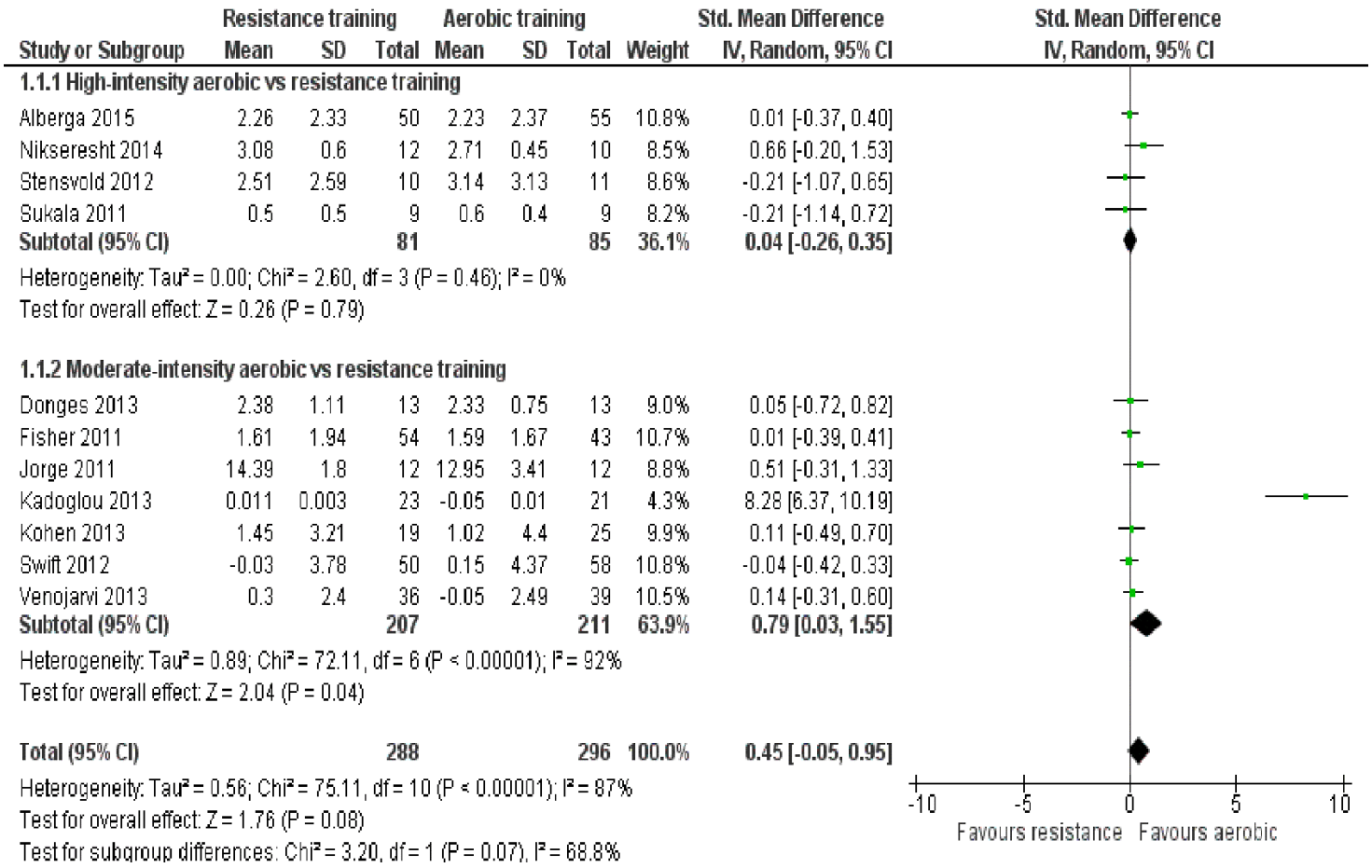

Figure 5. Florest plot: Difference between moderate-intensity aerobic vs resistance training on C-reactive Protein.

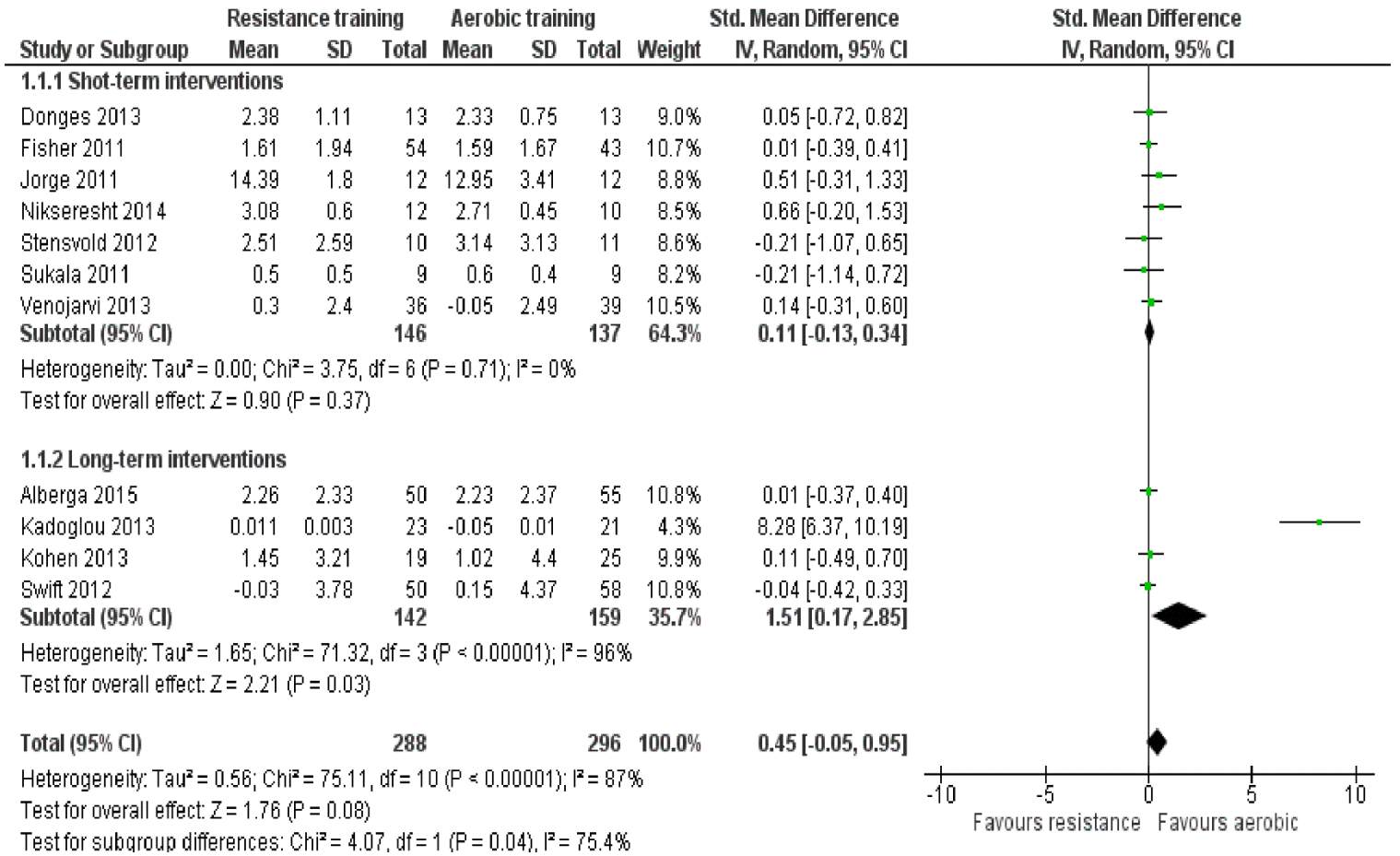

Figure 6. Florest plot: Difference in the reduction of C-reactive Protein between aerobic and resistance group (short vs long intenventions).

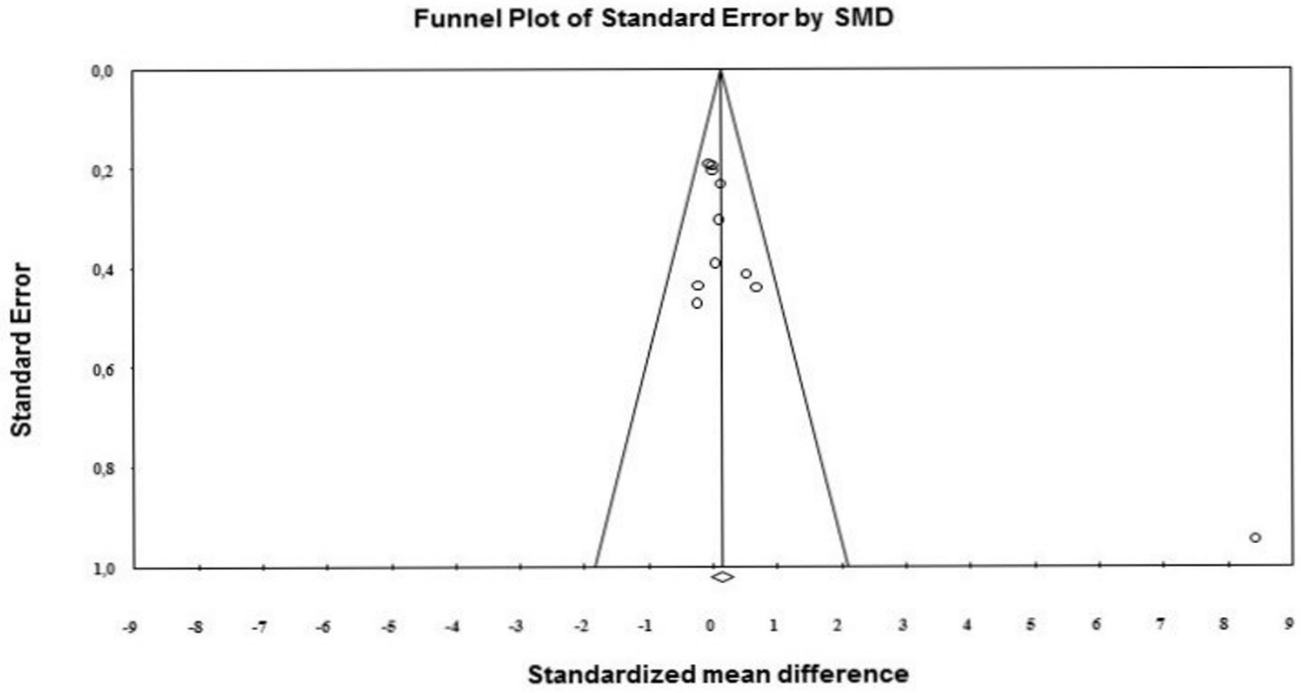

Figure 7. Funnel Plot for C-reactive protein outcome (11 studies).

Appendix 1. Search Strategy.

PubMed/MEDLINE and EMBASE (up to 7th of July, 2016)

1 – Trial' OR randomised OR randomized OR randomised controlled trial OR randomized controlled trial OR controlled trial OR clinical trial OR controlled clinical trial.

2 – Obesity OR cardiovascular disease OR cardiovascular diseases OR chronic diseases OR chronic disease OR chronic illness OR chronic illnesses OR chronically Ill OR metabolic diseases OR metabolic disease OR thesaurismoses OR thesaurismosis.

3 - High intensity interval exercise OR high intensity interval training OR aerobic interval exercise OR aerobic interval training OR endurance training OR aerobic exercise OR aerobic training.

4 - Strength exercise OR strength training OR resistance exercise OR resisted exercise OR resistance training OR resisted training.

5 - Inflammation status OR inflammation OR inflammatory markers OR c-reactive protein OR high-sensitivity C-reactive Protein OR cytokines.

6 - #1 AND #2 AND #3 AND #4 AND #5

7 – Animals OR animal.

8 - #6 NOT #7.

COCHRANE (up to 7th of July, 2016)

1 – Trial' OR randomised OR randomized OR randomised controlled trial OR randomized controlled trial OR controlled trial OR clinical trial OR controlled clinical trial.

2 – Obesity OR cardiovascular disease OR cardiovascular diseases OR chronic diseases OR chronic disease OR chronic illness OR chronic illnesses OR chronically Ill OR metabolic diseases OR metabolic disease OR thesaurismoses OR thesaurismosis.

3 - High intensity interval exercise OR high intensity interval training OR aerobic interval exercise OR aerobic interval training OR endurance training OR aerobic exercise OR aerobic training.

4 - Strength exercise OR strength training OR resistance exercise OR resisted exercise OR resistance training OR resisted training.

5 - Inflammation status OR inflammation OR inflammatory markers OR c-reactive protein OR high-sensitivity C-reactive Protein OR cytokines.

6 - #1 AND #2 AND #3 AND #4 AND #5

7 – Animals OR animal.

8 - #6 NOT #7

9 – Trials.

Appendix 2. Methodological quality of included studies

Studies	Eligibility criteria	Random allocation	Concealed allocation	Baseline comparability	Blind subjects	Blind therapists	Blind assessors	Adequate follow-up	Intention to treat analysis	Between group comparisons	Point estimates and variability	Total (0-10)
Alberga *et al* 2015	Yes	Yes	Yes	Yes	No	No	No	No	Yes	Yes	Yes	6
Nikseresht *et al.,* 2014	Yes	Yes	No	Yes	No	No	No	No	No	Yes	Yes	4
Kohen *et al.,* 2013	Yes	Yes	No	Yes	No	No	No	No	No	Yes	Yes	4
Kadoglou *et al.,* 2013	No	Yes	No	Yes	No	No	Yes	Yes	No	Yes	Yes	6
Venojärvi *et al.,* 2013	Yes	Yes	No	Yes	No	No	No	No	No	Yes	Yes	4
Donges *et al.,* 2013	No	Yes	No	Yes	No	No	No	Yes	No	Yes	Yes	5
Stensvolds *et al.,2012*	Yes	Yes	Yes	Yes	No	No	No	Yes	No	Yes	Yes	6
Swift *et al.,* 2012	No	Yes	No	Yes	No	No	Yes	No	No	Yes	Yes	5
Fisher *et al.,* 2011	No	Yes	No	Yes	No	No	No	No	No	Yes	Yes	4
Jorge *et al.,* 2011	Yes	Yes	No	Yes	No	No	No	Yes	No	Yes	Yes	5
Sukala *et al.,* 2011	Yes	Yes	No	Yes	No	No	No	No	No	Yes	Yes	4

Conclusões

A nova abordagem do treinamento aeróbio intervalado mostrou-se eficaz em reduzir níveis de inflamação crônica de baixo grau, pela diminuição sérica de proteína C reativa, bem como em aumentar a qualidade de vida de seus praticantes sem promover sobrecarga à nivel molecular.

Ainda, ao compararmos os efeitos do treinamento aeróbio *versus* treinamento resistido, por meio de meta-análise, o treinamento aeróbio demonstrou superioridade em melhorar o perfil inflamatório relacionado ao marcador fator de necrose tumoral alfa; no entanto, respostas similares foram observadas sobre os desfechos proteína C reativa e interleucina-6.

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ANEXO I – Normas para publicação do periódico: *Journal of Science and Medicine in Sport.*

Preparation of Manuscripts

Microsoft Word is the preferred software program. Use Arial or Times New Roman font, size eleven (11) point.

• Manuscript is double-spaced throughout (including title page, abstract, text, references, tables, and legends).
• Margins are 1 inch or 2.5 cm all around
• Include page and line numbers for the convenience of the peer reviewers.
• Number the pages consecutively, beginning with the title page as page 1 and ending with the Figure legend page.
• All headings (including the Title) should be in sentence-case only, not in capital letters.
• Sub-headings are generally not accepted. Incorporate into the text if required.
• Footnotes are not acceptable.
• Keep the use of tables, figures and graphs to a minimum.
• See notes on Tables, Figures, Formulae and Scientific Terminology at the end.

Word Count Limits

4000 word count limit (excluding title, abstract, tables/figures, figure legends, Acknowledgements, and References)

• Maximum number (combined) of tables and figures is 3
• Long tables should only be included as supplemental files and will be available online only
• Maximum number of references is 60
• A structured abstract of less than 250 words (not included in 4000 word count) should be included sticking as closely as possible to the following headings: Objectives, Design, Method,Results,and,Conclusions.

Structure of the Manuscript (in order):

1. Cover Letter - Every submission, regardless of category must include a letter stating:
•The category of article: Original Research or Review article.
•The sub-discipline: sports medicine, sports injury (including injury epidemiology and injury prevention), physiotherapy, podiatry, physical activity and health, sports science, biomechanics, exercise physiology, motor control and learning, sport and exercise psychology, sports nutrition, public health (as relevant to sport and exercise), rehabilitation and injury management, and others having an interdisciplinary perspective with specific applications to sport and exercise and its interaction with health.
• Sources of outside support for research (including funding, equipment and drugs) must be named.
• Financial support for the project must be acknowledged, or "no external financial support" declared.
• The role of the funding organisation, if any, in the collection of data, their analysis and interpretation, and in the right to approve or disapprove publication of the finished manuscript must be described in the Methods section of the text.

• When the proposed publication concerns any commercial product, either directly or indirectly,

the author must include a statement (1) indicating that he or she has no financial or other interest in the product or distributor of the product or (2) explaining the nature of any relation between himself or herself and the manufacturer or distributor of the product.

• Other kinds of associations, such as consultancies, stock ownership, or other equity interests or patent-licensing arrangements, also must be disclosed. Note: If, in the Editor's judgment, the information disclosed represents a potential conflict of interest, it may be made available to reviewers and may be published at the Editor's discretion; authors will be informed of the decision before/publication.

• The Ethical Guidelines that have been followed must be stated clearly. Provide the Ethics Committee name and approval number obtained for Human investigation.

• Authors must declare that manuscripts submitted to the Journal have not been published elsewhere or are not being considered for publication elsewhere and that the research reported will not be submitted for publication elsewhere until a final decision has been made as to its acceptability by the Journal.

2. Title Page (first page) should contain:

a. Title. Short and informative

b. Authors. List all authors by first name, all initials and family name

c. Institution and affiliations. List the name and full address of all institutions where the study described was carried out. List departmental affiliations of each author affiliated with that institution after each institutional address. Connect authors to departments using alphabetical superscripts.

d. Corresponding author. Provide the name and e-mail address of the author to whom communications, proofs and requests for reprints should be sent.

e. Word count (excluding abstract and references), the Abstract word count, the number of Tables, the number of Figures.

3. Manuscript (excluding all author details) should contain: (in order)

a. Abstract - must be structured using the following sub-headings: Objectives, Design, Methods, Results, and Conclusions. Avoid abbreviations and acronyms.

b. Keywords - provide up to 6 keywords, with at least 4 selected via the Index Medicus Medical Subject Headings (MeSH) browser list: Medical Subject Headings..These keywords should not reproduce words used in the paper title. c. Main body of the text.

For Original Research papers, text should be organised as follows:

i. Introduction - describing the (purpose of the study with a brief review of background.

ii. Methods - described in detail. Include details of the Ethics Committee approval obtained for Human investigation, and the ethical guidelines followed by the investigators. This section is not called Materials and Methods, and should not include subheadings. Do not use the term "subjects" - use terms such as "participants", "patients" or "athletes", etc.

iii. Results - concisely reported in tables and figures, with brief text descriptions. Do not include subheadings. Use small, non-italicized letter p for p-values with a leading zero, e.g. 0.05; Measurements and weights should be given in standard metric units. Do not replicate material that is in the tables or figures in the text.

iv. Discussion - concise interpretation of results. Cite references, illustrations and tables in numeric order by order of mention in the text. Do not include subheadings.

v.Conclusion.

vi. Practical Implications - 3 to 5 dot (bulleted) points summarising the practical findings derived from the study to the real-world setting of sport and exercise - that can be understood by a lay audience. Avoid overly scientific terms and abbreviations. Dot points should not include recommendations for further research.

vii. Acknowledgments - this section is compulsory. Grants, financial support and technical or other assistance are acknowledged at the end of the text before the references. All financial support for the project must be acknowledged. If there has been no financial assistance with the project, this must be clearly stated.

viii. References - authors are responsible for the accuracy of references.

ix. Tables - may be submitted at the end of the text file, on separate pages, one to each page.

x. Figure Legends - must be submitted as part of the text file and not as illustrations.

4. Figures - must be submitted as one or more separate files that may contain one or more images.

5. Supplementary material (if any) - tables or figures to be viewed online only.

ARTICLE STRUCTURE

Introduction

State the objectives of the work and provide an adequate background, avoiding a detailed literature survey or a summary of the results.

Material and methods

Provide sufficient detail to allow the work to be reproduced. Methods already published should be indicated by a reference: only relevant modifications should be described.

Results

Results should be clear and concise.

Discussion

This should explore the significance of the results of the work, not repeat them. A combined Results and Discussion section is often appropriate. Avoid extensive citations and discussion of published literature.

Conclusions

The main conclusions of the study may be presented in a short Conclusions section, which may stand alone or form a subsection of a Discussion or Results and Discussion section.

Appendices

If there is more than one appendix, they should be identified as A, B, etc. Formulae and equations in appendices should be given separate numbering: Eq. (A.1), Eq. (A.2), etc.; in a subsequent appendix, Eq. (B.1) and so on. Similarly for tables and figures: Table A.1; Fig. A.1, etc.

Essential title page information

• Title. Concise and informative. Titles are often used in information-retrieval systems. Avoid abbreviations and formulae where possible.

• Author names and affiliations. Please clearly indicate the given name(s) and family name(s) of each author and check that all names are accurately spelled. Present the authors' affiliation addresses (where the actual work was done) below the names. Indicate all affiliations with a lower-case superscript letter immediately after the author's name and in front of the appropriate address. Provide the full postal address of each affiliation, including the country name and, if available, the e-mail address of each author.
• Corresponding author. Clearly indicate who will handle correspondence at all stages of refereeing and publication, also post-publication. Ensure that the e-mail address is given and that contact details are kept up to date by the corresponding author.
• Present/permanent address. If an author has moved since the work described in the article was done, or was visiting at the time, a 'Present address' (or 'Permanent address') may be indicated as a footnote to that author's name. The address at which the author actually did the work must be retained as the main, affiliation address. Superscript Arabic numerals are used for such footnotes.

Abstract
A concise and factual abstract is required. The abstract should state briefly the purpose of the research, the principal results and major conclusions. An abstract is often presented separately from the article, so it must be able to stand alone. For this reason, References should be avoided, but if essential, then cite the author(s) and year(s). Also, non-standard or uncommon abbreviations should be avoided, but if essential they must be defined at their first mention in the abstract itself.

Keywords
Immediately after the abstract, provide a maximum of 6 keywords, using British spelling and avoiding general and plural terms and multiple concepts (avoid, for example, 'and', 'of'). Be sparing with abbreviations: only abbreviations firmly established in the field may be eligible. These keywords will be used for indexing purposes.

Abbreviations
Define abbreviations that are not standard in this field in a footnote to be placed on the first page of the article. Such abbreviations that are unavoidable in the abstract must be defined at their first mention there, as well as in the footnote. Ensure consistency of abbreviations throughout the article.

Acknowledgements
Collate acknowledgements in a separate section at the end of the article before the references and do not, therefore, include them on the title page, as a footnote to the title or otherwise. List here those individuals who provided help during the research (e.g., providing language help, writing assistance or proof reading the article, etc.).

Formatting of funding sources
List funding sources in this standard way to facilitate compliance to funder's requirements:

Funding: This work was supported by the National Institutes of Health [grant numbers xxxx, yyyy]; the Bill & Melinda Gates Foundation, Seattle, WA [grant number zzzz]; and the United States Institutes of Peace [grant number aaaa].

If no funding has been provided for the research, please include the following sentence: This

research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Nomenclature and units

Follow internationally accepted rules and conventions: use the international system of units (SI). If other quantities are mentioned, give their equivalent in SI. You are urged to consult IUPAP: Symbols, Units, Nomenclature and Fundamental Constants in Physics for further information.

Footnotes

Footnotes should be used sparingly. Number them consecutively throughout the article. Many word processors can build footnotes into the text, and this feature may be used. Otherwise, please indicate the position of footnotes in the text and list the footnotes themselves separately at the end of the article. Do not include footnotes in the Reference list.

Artwork

Electronic artwork

General points

• Make sure you use uniform lettering and sizing of your original artwork.
• Embed the used fonts if the application provides that option.
• Aim to use the following fonts in your illustrations: Arial, Courier, Times New Roman, Symbol, or use fonts that look similar.
• Number the illustrations according to their sequence in the text.
• Use a logical naming convention for your artwork files.
• Provide captions to illustrations separately.
• Size the illustrations close to the desired dimensions of the published version.
• Submit each illustration as a separate file.

Human and animal rights

If the work involves the use of human subjects, the author should ensure that the work described has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; Uniform Requirements for manuscripts submitted to Biomedical journals. Authors should include a statement in the manuscript that informed consent was obtained for experimentation with human subjects. The privacy rights of human subjects must always be observed

Figure captions

Ensure that each illustration has a caption. Supply captions separately, not attached to the figure.A caption should comprise a brief title (not on the figure itself) and a description of the illustration. Keep text in the illustrations themselves to a minimum but explain all symbols and abbreviations used.

Tables

Please submit tables as editable text and not as images. Tables can be placed either next to the relevant text in the article, or on separate page(s) at the end. Number tables consecutively in accordance with their appearance in the text and place any table notes below the table body. Be sparing in the use of tables and ensure that the data presented in them do not duplicate results

described elsewhere in the article. Please avoid using vertical rules and shading in table cells.

References

Citation in text.

Please ensure that every reference cited in the text is also present in the reference list (and vice versa). Any references cited in the abstract must be given in full. Unpublished results and personal communications are not recommended in the reference list, but may be mentioned in the text. If these references are included in the reference list they should follow the standard reference style of the journal and should include a substitution of the publication date with either 'Unpublished results' or 'Personal communication'.

Citation of a reference as 'in press' implies that the item has been accepted for publication.

Reference links

Increased discoverability of research and high quality peer review are ensured by online links to the sources cited. In order to allow us to create links to abstracting and indexing services, such as Scopus, CrossRef and PubMed, please ensure that data provided in the references are correct. Please note that incorrect surnames, journal/book titles, publication year and pagination may prevent link creation. When copying references, please be careful as they may already contain errors. Use of the DOI is encouraged.

A DOI can be used to cite and link to electronic articles where an article is in-press and full citation details are not yet known, but the article is available online. A DOI is guaranteed never to change, so you can use it as a permanent link to any electronic article. An example of a citation using DOI for an article not yet in an issue is: VanDecar J.C., Russo R.M., James D.E., Ambeh W.B., Franke M. (2003). Aseismic continuation of the Lesser Antilles slab beneath northeastern Venezuela. Journal of Geophysical Research, http://dx.doi.org/10.1029/2001JB000884i.

Web references

As a minimum, the full URL should be given and the date when the reference was last accessed. Any further information, if known (DOI, author names, dates, reference to a source publication, etc.), should also be given. Web references can be listed separately (e.g., after the reference list) under a different heading if desired, or can be included in the reference list.

References in a special issue

Please ensure that the words 'this issue' are added to any references in the list (and any citations in the text) to other articles in the same Special Issue.

Reference Style

• References should be numbered consecutively in un-bracketed superscripts where they occur in the text, tables, etc, and listed numerically (e.g. "1", "2") at the end of the paper under the heading "References".

• For Original Research papers, no more than three references should be used to support a specific point in the text.

• All authors should be listed where there are three or fewer. Where there are more than three, the reference should be to the first three authors followed by the expression "et al".

• Book and journal titles should be in italics.

• Conference and other abstracts should not be used as references. Material referred to by the phrase "personal communication" or "submitted for publication" are not considered full references and should only be placed in parentheses at the appropriate place in the text (e.g.,

(Hessel 1997 personal communication). References to articles submitted but not yet accepted are not encouraged but, if necessary, should only be referred to in the text as "unpublished data".
• Footnotes are unacceptable.
• Book references:Last name and initials of author, chapter title, chapter number, italicised title of book, edition (if applicable), editor, translator (if applicable), place of publication, publisher, year of publication.Example:Wilk KE, Reinold MM, Andrews JR. Interval sport programs for the shoulder, Chapter 58, in The Athlete's Shoulder, 2nd ed., Philadelphia, Churchill Livingstone, 2009
• Journal references:
Last name and initials of principal author followed by last name(s) and initials of co-author(s), title of article (with first word only starting in capitals), abbreviated and italicised title of journal, year, volume (with issue number in parenthesis if applicable), inclusive pages.

Supplementary material
Supplementary material such as applications, images and sound clips, can be published with your article to enhance it. Submitted supplementary items are published exactly as they are received (Excel or PowerPoint files will appear as such online). Please submit your material together with the article and supply a concise, descriptive caption for each supplementary file. If you wish to make changes to supplementary material during any stage of the process, please make sure to provide an updated file. Do not annotate any corrections on a previous version. Please switch off the 'Track Changes' option in Microsoft Office files as these will appear in the published version.