ESCOLA BAHIANA DE MEDICINA E SAÚDE PÚBLICA
PROGRAMA DE PÓS-GRADUAÇÃO EM MEDICINA E SAÚDE HUMANA

DJEYNE SILVEIRA WAGMACKER

EXERCÍCIO FÍSICO E ÁCIDOS GRAXOS: ALTERAÇÕES METABÓLICAS, INFLAMATÓRIAS E DE ESTRESSE OXIDATIVO.

TESE DE DOUTORADO

Salvador-Bahia

2018

DJEYNE SILVEIRA WAGMACKER

EXERCÍCIO FÍSICO E ÁCIDOS GRAXOS: ALTERAÇÕES METABÓLICAS, INFLAMATÓRIAS E DE ESTRESSE OXIDATIVO.

Tese apresentada ao curso de Pós-graduação em Medicina e Saúde Humana da Escola Bahiana de Medicina e Saúde Pública para obtenção do título de Doutora em Saúde Humana

Orientadores: Prof. Dra. Ana Marice Teixeira Ladeia
Prof. Dr. Luiz Erlon Rodrigues

Salvador-Bahia
2018

Ficha Catalográfica elaborada pelo Sistema Integrado de Bibliotecas da EBMSP

W111 Wagmacker, Djeyne Silveira
Exercício físico e ácidos graxos: alterações metabólicas, inflamatórias e de estresse oxidativo. Djeyne Silveira Wagmacker/. – 2018.
121f.: il. color.; 30cm.

Orientadores: Profa. Dra. Ana Marice Teixeira Ladeia
Prof. Dr. Luiz Erlon Rodrigues

Doutora em Medicina e Saúde Humana. 2018.

Inclui bibliografia

1. Obesidade. 2.Atividade Motora. 3.Ácidos graxos.4. Metabolismo.5. Estresse oxidativo I. Título.

CDU: 616-056.2

DJEYNE SILVEIRA WAGMACKER

"EXERCÍCIO FÍSICO E ÁCIDOS GRAXOS: ALTERAÇÕES METABÓLICAS, INFLAMATÓRIAS E DE ESTRESSE OXIDATIVO"

Tese apresentada à Escola Bahiana de Medicina e Saúde Pública, como requisito parcial para a obtenção do Título de Doutora em Medicina e Saúde Humana.

Salvador, 25 de abril de 2018.

BANCA EXAMINADORA

Profª. Dr.ª Maria de Fátima Dias Costa
Doutora em Neuroquimica
Universidade Federal da Bahia, UFBA

Prof. Dr. Fabiano Leichsenring Silva
Doutor em Ciências Biológicas
Faculdade Adventista da Bahia, FADBA

Prof. Dr. Wellington dos Santos Silva
Doutor em Patologia molecular
Faculdade Adventista da Bahia, FADBA

Prof. Dr. Luis Cláudio Lemos Correia
Doutor em Medicina e Saúde Humana
Escola Bahiana de Medicina e Saúde Pública, EBMSP

Prof. Dr. Armênio Costa Guimarães
Doutor em Fellowship Em Cardiologia
Escola Bahiana de Medicina e Saúde Pública, EBMSP

Dedico este trabalho à meu esposo Kendall Ferreira e minhas filhas Eduarda e Giovana. Nada seria possível e tampouco valeria a pena sem vocês ao meu lado.

AGRADECIMENTOS

À Deus por sua constante permanência ao meu lado, por me dar a certeza de que não estive só nos diversos momentos e por me dar a perseverança para continuar insistindo sempre. Obrigada por me oportunizar realizar este sonho.

À Meu esposo (Anjo), como assim o chamo, por ser um anjo que Deus colocou em minha vida para me cuidar e estarmos juntos sempre. Obrigada por todo amor desprendido, por me fazer rir mesmo em circunstâncias adversas, por me estimular sempre e acreditar mais em mim do que eu mesma. Obrigada por sonharmos juntos e por ser meu grande incentivador. Por me dar a certeza de que se nada nesta vida der certo, eu já tenho o que de melhor poderia levar dela, você. E como você mesmo diz, esta vitória não é minha, é nossa. Obrigada meu Anjo.

À Minhas Filhas Eduarda e Giovana, por todo amor incondicional, por compreenderem minhas ausências, por serem os motivos de minha superação, por me esperarem retornar para casa a cada sexta-feira com os sorrisos mais puros e sinceros, por me receberem com os melhores abraços e pelas palavras mais doces e sinceras: "mamãe estava com saudade, amo você". A vocês a minha eterna gratidão simplesmente por existirem.

À minha Orientadora, Dra. Ana Marice a minha profunda gratidão por tamanha competência, profissionalismo, atenção, acessibilidade e compromisso. Obrigada por ter sempre um caminho a indicar, por responder sempre as minhas dúvidas, por ter sido sempre acessível, por nunca ter deixando de responder um email, watsapp ou retornar um telefonema. Obrigada por ter sido além de orientadora uma amiga e conselheira. Obrigada por acreditar sempre que tudo daria certo e por me estimular a cada momento. Obrigada pelos 6 anos de trabalho juntas, entre mestrado e doutorado. Tenho certeza de que a maior riqueza de todo este processo foi ter tido o privilégio de conhecer e trabalhar com alguém de tamanha grandeza. A você a minha eterna gratidão.

Ao meu co-orientador, Dr. Luiz Erlon. Obrigada por me tranquilizar sempre, por me apresentar o mundo complexo dos ácidos graxos com tamanha profundidade. Obrigada por fazer o complexo parecer simples. Conhecê-lo foi um grande privilégio. Minha profunda gratidão.

À FADBA, por todo apoio sempre ofertado, por acreditar em mim e por financiarem este sonho. Sou eternamente grata por todo esforço e compromisso com minha formação.

Ao laboratório de neurociência da UFBA em nome da Dra Maria de Fátima Dias

Costa. Obrigada por cederem o espaço para que pudessemos desenvolver parte deste estudo. Obrigada por tamanha cordialidade e simpatia e por serem tão solícitos às nossas necessidades. Obrigada por nos receberem como se estivéssemos em nossa casa durante aproximadamente 18 meses. Nossa profunda admiração e respeito a este laboratório e toda a sua equipe.

Ao Laboratório de Fisiologia da UFRGS em nome da Dra. Adriane Bello Klein. Obrigada por tamanha cordialidade e receptividade, por completarem parte de um sonho. Nosso muito obrigado pela disposição em realizarem todas as análises de estresse oxidativo e nos atenderem em nossas inúmeras dúvidas.

À equipe técnica do projeto, obrigada por tamanho compromisso. Por se empenharem tanto quanto eu com o cumprimento de nossas metas para que pudessemos terminar em tempo hábil este experimento.

Aos amigos Jefferson Petto e Fabiano Leinschering. Obrigada pela experiência de pesquisa compartilhada, por tornarem o caminho mais leve e breve, por pensarmos juntos em soluções viáveis e por todos os sábios conselhos. Obrigada acima de tudo por desfrutarmos de tão saudável amizade. Minha profunda admiração pelos pesquisadores que se tornaram e pela amizade que compartilhamos.

Aos alunos de PIBIC a minha profunda admiração a gratidão, sem a participação de vocês este trabalho não seria possível. Muitos estiveram envolvidos ao longo destes quatro anos de pesquisa e sou imensamente grata pela participação de cada um, mas não poderia deixar de citar pessoas como Darlei, Fabiane, Luana, Elaine, Jessica, Jackeline e Sindy. Meu muito obrigada por todo empenho e compromisso com esta pesquisa.

Aos meus familiares e amigos, meu muito obrigada por esterem sempre presente, pelas inúmeras orações e pela grande torcida para que esta trajetória fosse exitosa.

"Eu não sou quem eu gostaria de ser; eu não sou quem eu deveria ser, eu não sou quem eu poderia ser ainda. Mas graças a Deus eu não sou mais quem eu era."

Martin Luther King.

RESUMO

INTRODUÇÃO: O excesso de peso corporal é fator predisponente para doenças cardiometabólicas. Alimentação inadequada relaciona-se ao excesso de tecido adiposo, ativando vias inflamatórias induzindo a estresse oxidativo e lesão vascular. O exercício físico têm sido apontado como ferramenta de prevenção e controle, porém seus resultados permanecem contraditórios. **OBJETIVO:** O objetivo primário foi testar a hipótese de que o exercício físico de maneira aguda altera os ácidos graxos do soro de indivíduos com aumento do peso corporal. Nos objetivos secundários, foi investigado o efeito de uma sessão de exercício físico de baixa intensidade sobre a glicemia, os valores lipídicos e o estresse oxidativo de mulheres com excesso de peso corporal. **MÉTODOS:** Esta tese foi realizada como fruto da parceria científica entre a Escola Bahiana de Medicina e Saúde Pública, a Faculdade Adventista da Bahia, a Universidade Federal da Bahia e a Universidade Federal do Rio Grande do Sul. Mulheres com excesso de peso foram recrutadas do ambulatótio de cardiologia da Faculdade Adventista da Bahia, as amostras de sangue foram coletadas dos pacientes antes e 12h após o exercício físico. O soro foi congelado a -80ºC e posteriormente analisado. Quanto ao objetivo primário, os ácidos graxos foram medidos em cromatografia gasosa, com as amostras de soro previamente transesterificadas. Quanto aos objetivos secundários, foram feitas dosagens do perfil glicêmico, e de estresse oxidativo, sendo estes analisados a partir das espécies reativas de ácido tiobarbitúrico (TBARS), carbonilas e sulfidrilas. **RESULTADOS:** cinco artigos científicos compõem a tese. Como resultado primário, o exercício físico de baixa intensidade não foi capaz de modificar os ácidos graxos de cadeia média. Em relação aos objetivos secundários o exercício provocou redução da glicemia não modificando a resposta lipídica, reduziu a peroxidação lipídica, não interferindo na peroxidação proteica e no fator antioxidante e em mulheres com excesso de peso, o consumo de gorduras poli-insaturada esteve relacionada a menor inflamação subclínica neste grupo de mulheres. **CONCLUSÃO:** O exercício físico de baixa intensidade foi capaz de diminuir os valores glicêmicos e diminuir a peroxidação lipídica, porém não interferiu na resposta lipídica, antioxidante e na peroxidação proteica. O consumo de gordura poliinsaturada reduz a inflamação subclínica em mulheres com excesso de peso.

Palavras-chave: Obesidade. Atividade Motora. Ácidos graxos. Metabolismo. Estresse oxidativo.

ABSTRACT

INTRODUCTION: Excess body weight is a predisposing factor for cardiometabolic diseases. Inadequate feeding relates to excess adipose tissue, activating inflammatory pathways inducing oxidative stress and vascular injury. Physical exercise has been pointed out as a prevention and control tool, but its results remain contradictory. **OBJECTIVE:** The primary objective was to test the hypothesis that acute exercise modifies the serum fatty acids of individuals with increased body weight. In the secondary objectives, the effect of a physical exercise session low intensity on glycemia, lipid values and oxidative stress of overweight women was investigated. **METHODS:** This thesis was carried out as a result of the scientific partnership between the Bahian School of Medicine and Public Health, the Adventist College of Bahia, the Federal University of Bahia and the Federal University of Rio Grande do Sul. Overweight women were recruited from the cardiology ward of the Adventist College of Bahia, blood samples were collected from the patients before and 12h after physical exercise. The serum was frozen at -80 ° C and then analyzed. Regarding the primary objective, the fatty acids were measured in gas chromatography, with serum samples previously transesterified. Regarding the secondary objectives, dosages of the glycemic profile and of oxidative stress were made, being these analyzed from the reactive species of thiobarbituric acid (TBARS), carbonyls and sulfhydryl. **RESULTS:** Five scientific articles compose the thesis. As a primary outcome, low intensity physical exercise was not able to modify medium chain fatty acids. Regarding the secondary objectives, the exercise caused a reduction of glycemia, not modifying the lipid response, reduced lipid peroxidation, and did not interfere with protein peroxidation and antioxidant factor, and in overweight women, the consumption of polyunsaturated fats was related to lower subclinical inflammation. **CONCLUSION:** The response to low intensity physical exercise is able to decrease glycemic values and decrease lipid peroxidation, but does not interfere with lipid response, antioxidant and protein peroxidation. The consumption of polyunsaturated fat reduces subclinical inflammation in overweight women.

Key words: Obesity. Motor activity. Fatty acids. Metabolism. Oxidative stress.

LISTA DE ABREVIATURAS

Acil-CoA	Acetilcoenzima A
AG	Ácidos Graxos
ALA	Ácido alfa-linolênico
AMP	Adenosina Monofosfato
AMPK	Proteína Quinase Ativada por Adenosina Monofosfato
ATP	Adenosina Trifosfato
CAT	Catalase
CNPQ	Conselho Nacional de Desenvolvimento Científico e Tecnológico
CT	Colesterol Total
DAC	Doença Arterial Coronariana
DHA	Ácido docosahexenóico
DM	Diabetes Mellitus
DM2	Diabetes Mellitus tipo 2
DNPH	Dinitrofenilhidrazina
DP	Desvio Padrão
DTNB	Ácido 2,2-dinitro-5,5-ditiodibenzoico
EDTA	Ácido Etilenodiaminotetracético
eNOS	Óxido Nítrico Sintetase endotelial
EPA	Ácido icosapentenóico
ERO	Espécies Reativas de Oxigênio
EROs	Espécies Reativas de Oxigênio
GC	Grupo Controle
GCIV	Grupo com inflamação vascular
GE	Grupo exercício
GPX	Glutationa peroxidase

GSIV	Grupo sem inflamação vascular
HDLc	Lipoproteína de alta densidade
IC	Intervalo de Confiança
ICAM-1	Molécula de adesão intercelular - 1
IH	Índice do Homa
IIQ	Intervalo interquartil
IL-1	Interleucina-1
IL-6	Interleucina-6
IMC	Índice de massa corporal
IPAQ	Questionário Internacional de Atividade Física
IRS	Receptores de Insulina
Kcal	Quilocaloria
LA	Ácido linoléico
LDL-c	Lipoproteína de Baixa Densidade
LHS	Lipase sensível a hormônio
LpRT	Lipoproteína Ricas em Triglicerídeos
MDA	Malondialdeído
mRNA	RNA Mensageiro
NO	Óxido Nítrico
NO_2	Dióxido de Nitrogênio
O_2^-	Ânio superóxido
OMS	Organização Mundial de saúde
$ONOO^-$	Ânion Peroxinitrito
OR	Odds Ratio
PCR	Proteína C Reativa
PKC	Proteína Quinase C
R	Correlação

RCQ	Relação Cintura Quadril
RI	Resistência Insulínica
RONS	Espécies de Nitrogênio
ROS	Espécies Reativas do Oxigênio
SDS	Sodium Dodecil Sulfate
SOD	Superóxido Desmutase
SPSS	Pacote Estatístico para Ciências Sociais
TBARS	Espécies Reativas de Ácido Tiobarbitúrico
TCA	Ácido Tricoloacético
TG	Triglicerídeos
TG/HDL	Razão entre Triglicerídeos e Lipoproteínas de alta densidade
TGIM	Triglicerídeo Intramiocelular
TGLA	Triglicerídeo Lipídico Adiposo
TNF-α	Fator de Necrose Tumoral – Alfa
VCAM-1	Molécula de adesão da célula vascular – 1
VLDL	Lipoproteína de muito baixa densidade
VO_2 máx	Volume de Oxigênio Máximo

SUMÁRIO

1 INTRODUÇÃO

As doenças cardiovasculares continuam sendo a principal causa de morbimortalidade no mundo, apesar das melhorias nos resultados destes indicadores[1]. As taxas da Doença Arterial Coronariana (DAC) reduziram em muitos países da Europa[2], no entanto, fatores de risco como obesidade têm aumentado substancialmente em muitos países. Não são apenas fatores de risco prevalentes que causam preocupações em relação a DAC, mas também uma baixa implementação de medidas preventivas para diminuir dieta de baixa qualidade, inatividade física e tabagismo[3].

Grande parte dos distúrbios cardiovasculares tem origem na aterosclerose, caracterizada por alterações na íntima, representadas por acúmulo de lipídeos, componentes do sangue, células, material intercelular e carboidratos[4].

As dietas representam exposições diversas podendo, a depender do padrão alimentar, influenciar em aspectos nocivos e efeitos cumulativos, gerando resultados deletérios à saúde como alterações de marcadores inflamatórios sistêmicos, distúrbios metabólicos e doenças cardiovasculares[5].

Os lipídios sempre estiveram presentes nas dietas, a gordura consumida é predominantemente composta de ácidos graxos (AG) e glicerol. A maior parte dos AG em humanos são de cadeia longa divididos em saturados e insaturados que podem apresentar configuração cis ou trans[6]. A composição dos AG provenientes da dieta é um fator importante uma vez que provocam alterações metabólicas distintas[7].

Atualmente têm sido observado um aumento do consumo de gordura trans pelos indivíduos, o que têm despertado interesse da comunidade científica uma vez que o consumo de ácidos graxos trans tem sido relacionado a aumento do risco de doenças coronarianas[8], alterações nas lipoproteínas plasmáticas e triglicerídeos, aumento do risco de Diabetes Mellitus[9], elevação de marcadores séricos inflamatórios[5], aumento do estresse oxidativo e disfunção endotelial[10].

Percebe-se um consenso na indicação da prática de exercício como mecanismo protetor de disfunções cardiovasculares e metabólicas porém o que têm sido observado em diferentes estudos é que os resultados são conflitantes, não sendo evidenciada clareza nos efeitos desta terapêutica isolada sobre o sistema metabólico e vascular[3].

Estudos também sugerem que o exercício físico, de maneira aguda, promove modificação de gens transportadores de ácidos graxos, precedendo aumentos na expressão de

RNAm[11]. Isso permite a entrega ótima de ácidos graxos aos transportadores, e maior metabolização dos mesmos, apesar de este último ainda não ter sido testado[12].

Diferentes estudos avaliaram os efeitos agudos e crônicos do exercício físico sobre variáveis metabólicas[13-14] e em muitos estudos observou-se que o exercício físico foi eficiente terapia reguladora do perfil lipídico e glicêmico[13-15]. Porém os efeitos de uma única sessão de exercício físico tem também sido objeto de estudo de algumas pesquisas, uma vez que existem fortes contrapontos entre os estudos. Em alguns evidencia-se efeitos positivos, enquanto, outros não apontam modificações do perfil metabólico com apenas uma sessão[16 - 17].

Dado que a atividade glicolítica pode ser alterada pela obesidade e que a utilização de lipídios é modulada pela disponibilidade de ácidos graxos no plasma parece interessante abordar os lipídios, como um nutriente energético alternativo para os músculos que trabalham durante o exercício de baixa a moderada intensidade em indivíduos obesos. Estudos anteriores revelam que os níveis de AG plasmáticos são mais elevados nesta população e esta disponibilidade aumentada de AG circulante pode potencialmente atender às necessidades oxidativas dos músculos que trabalham durante o exercício[18].

Em obesos, durante o exercício, o metabolismo anaeróbio encontra-se limitado por diversos fatores, entre eles a presença de fadiga prematura e a redução da ativação das fibras musculares do tipo II (fibras brancas). Neste contexto a atividade aeróbia encontra-se favorecida e o metabolismo dos ácidos graxos, passa a ser uma opção de fornecimento de energia mais viável. Estudos sugerem que em obesos as respostas metabólicas de mobilização dos ácidos graxos parece ser favorecida com a atividade aeróbica, porém as respostas ainda não são conclusivas[18].

De maneira geral, os estudos sugerem o exercício físico interfira positivamente na redução do estresse oxidativo. A compreensão do estresse oxidativo parte do entendimento de que a oxidação é parte fundamental metabolismo aeróbio humano e que a produção de radicais livres ocorre naturalmente ou por alguma disfunção biológica. Quando ocorre o excesso da produção de radicais livres o organismo dispõe de um eficiente sistema antioxidante. Quando ocorre um desequilíbrio entre o sistema pro-oxidante e antioxidante, com predomínio do primeiro, é estabelecida uma situação de estresse oxidativo. Esta situação apresenta efeitos nocivos ao organismo, entre eles a lipoperoxidação proteica da membrana[19].

O exercício físico de baixa a moderada intensidade também tem sido apontado como um fator positivo no controle do estresse oxidativo, porém os resultados ainda são

divergentes. Alguns estudo têm sinalizado que o exercício agudo pode induzir a um estado de estresse oxidativo[19-20] outros estudos sugerem que o estresse oxidativo induzido pelo exercício possa promover a expressão da defesa antioxidante e as respostas adaptativas ao treinamento[21]. Alguns outros estudos têm sugerido que a atividade física pode reduzir o estresse oxidativo[22] .

Quanto aos efeitos da intensidade do exercício sobre o estado redox os resultados permanecem conflitantes[23]. Em alguns estudos o exercício de alta intensidade tem sido sinalizados como responsável por aumentar o consumo de oxigênio e estimular mitocôndrias e linfócitos, resultando na produção de oxigênio reativo e espécies de nitrogênio (RONS)[24-25]. Outros estudos sugerem que o exercício de alta intensidade pode reduzir a produção de espécies reativas do oxigênio[26]. O exercício de baixa a moderada intensidade têm sido sugerido como positivo em alguns estudo na redução do estresse oxidativo[27] e em outros estudos seu efeito estressor parece estar presente[28].

Apesar de ainda carecermos de respostas mais concretas, de maneira geral a literatura sugere uma resposta benéfica do exercício físico sobre o estado inflamatório. A inflamação é considerada um central no desenvolvimento, progressão e desfecho da aterosclerose. Esse processo inflamatório causa mudanças estruturais e funcionais na parede dos vasos sanguíneos, que conduzem a disfunção endotelial. Diversos fatores como o padrão alimentar e metabólico dos indivíduos podem influenciar o estado inflamatório. Sabe-se que o aumento do tecido adiposo relaciona-se a um estado de inflamação subclínica com aumento de marcadores séricos inflamatórios como no caso a proteína C reativa.[29]

A atividade física em alguns estudos, esteve relacionado ao aumento da resposta antiinflamatória principalmente em indivíduos jovens, porém os mecanismos que explicam este real efeito ainda não estão totalmente esclarecidos na literatura.[30]

A partir das necessidades de maiores esclarecimentos quanto aos reais efeitos subagudos do exercício físico de baixa intensidade sobre o metabolismo de ácidos graxos, a resposta metabólica, a inflamação e o estresse oxidativo em mulheres com aumento do peso corporal foi desenvolvido este ensaio clínico randomizado.

2 OBJETIVOS

2.1 Primário

Testar a hipótese de que o exercício físico de maneira aguda altera os ácidos graxos de cadeia média e longa do soro de indivíduos com aumento do peso corporal. **Artigos # 2**

2.2. Secundários

Testar a hipótese de que uma sessão de exercício físico baseado no gasto calórico pode modificar de forma aguda a glicemia e os valores lipídicos de mulheres com excesso de massa corporal. **Artigo # 3**

Testar as hipóteses de que uma sessão de exercício físico de baixa a moderada intensidade altera o stress oxidativo após 12 horas em mulheres com aumento do peso corporal. **Artigo # 4**

2.3 Terciário

Testar a associação entre inflamação subclínica e fatores alimentares e metabólicos em mulheres com excesso de peso. **Artigo # 5**

3 REVISÃO DE LITERATURA

3.1 ARTIGO 1 - Fatty acid metabolism, secondary complications and effects of physical exercise: an integrative review.

International Journal Cardiovascular Science. **Em revisão**

FATTY ACID METABOLISM, SECONDARY COMPLICATIONS AND EFFECTS OF PHYSICAL EXERCISE: AN INTEGRATIVE REVIEW

ABSTRACT

INTRODUCTION: Diet is a complicated set of exposures that frequently interact, and whose cumulative effects influence the results of health. This includes effects on systemic inflammation markets in metabolic disturbances and cardiovascular diseases. Various studies have been presented relating the effect of physical exercise on lipids, however, the results are still controversial.

OBJECTIVE: To describe fatty acid metabolism and the effect of physical exercise on secondary complications.

METHODS: An integrative review was conducted on subjects in the Medline, pubmed, web of science and scopus databases, published up to the year 2017.

RESULTS: Fatty acids, depending on their biochemical characteristics and spatial configuration, have differentiated effect on cardiovascular health, however, studies still present contradictory results about the therapeutic use of certain fatty acids. Physical activity appears to benefit fatty acid metabolism and attenuate the complications secondary to the consumption of certain fatty acids, and potentializes the positive effects of distinct fatty acids.

CONCLUSION: However, variants of physical activity, such as intensity, duration, time of observation of effects of the results, limit the authors to concluding, with a certain degree of certainty, about the effect of physical exercise on fatty acids and secondary complications, since the studies in the literature continue to be contradictory.

KEYWORDS: Acids Fatty, Exercise, inflammation, oxidative stress

FATTY ACID METABOLISM, SECONDARY COMPLICATIONS AND EFFECTS OF PHYSICAL EXERCISE: AN INTEGRATIVE REVIEW

INTRODUCTION

Cardiovascular diseases continue to be the main cause of morbidity and mortality in the world, in spite of improvements in the results(1-2). However, risk factors such as obesity and diabetes mellitus(DM) have increased substantially and increased the inequalities among countries. Not only the prevalent risk factor cause concern about these diseases, but also the low level of implementation of preventive measures, such as low quality diet and physical inactivity(3).

A large portion of the cardiovascular disturbances have their origin in atherosclerosis, characterized by changes in the intima, represented by accumulation of lipids, components of the blood, cells, intercellular matter and carbohydrates(4).

Lipids have always been present in diets. Diet is a complicated set of exposures that frequently interact, and whose cumulative effects influence the results of health. This includes effects on systemic inflammation markets in metabolic disturbances and cardiovascular diseases(5).

The fat consumed is composed of fatty acids(FA) and glycerol. The larger part of FAs in humans are of the long chain type, divided into saturated and unsaturated types that may present a cis or trans configuration(6). The composition of FAs coming from the diet is an important factor, because they cause different metabolic changes(7).

At present, an increase in trans fat consumption by individuals has been observed. This has aroused the interest of the scientific community, because the consumption of trans fatty acids has been related to increased risk of coronary diseases(8), changes in plasma lipoproteins and triglycerides, increased risk for Diabetes Mellitus(9), elevation of Serum inflammatory markers(5) oxidative stress and endothelial dysfunction markers, as well as worsened nitric oxide-mediated vasodilator response(10). The physical-chemical characteristics of fatty acids, such a melting point, carbon chain size, presence of double bonds and geometric configurations are important aspects that may interfere in the absorption of fatty acids by tissues, especially the adipose and vascular types, and the development of health problems.

Various studies have been presented relating the effect of physical exercise on lipids, however, the results are still controversial(11-14). Over the last few decades it has been

possible to observe growing evidence that acute physical exercise could have an acute beneficial influence on the lipid profile(15-16). The difficulty with analyses and interpretation of these studies lies in the use of different physical activity protocols established.

FATTY ACIDS: DEFINITION AND CLASSIFICATION

Lipids are distinct elements among them, presenting different chemical and functional characteristics. Fatty acids, the constituent elements of lipids, are present in any lipid structure.

Fatty acids are organic components that contain carbon and hydrogen in their molecules. Depending on the type of combination among fatty acids and their constituents, they will form different types of lipids. Based on these combinations, they may be classified as simple or complex types. Simple lipids are those in which the fatty acid combines with only one other element (e.g.: triglycerides), whereas, complex lipids are those in which the fatty acid combines with more than one element (e.g.: lipoproteins)(17).

Among the characteristics that distinguish the fatty acids is the size of the carbonated chain. Fatty acids may also be classified as short and long chain types. The short chain type have between 4 and 16 carbon molecules, and when they are not supplied by the diet, they are synthesized, mainly in the cytoplasm of hepatic and adipose tissue cells. The long chain fatty acids have 16 or more carbon molecules, and when they are not supplied by the diet, they are formed by elongation of pre-existent fatty acids(18). In addition, fatty acids may be classified based on the type of bond among their molecules, differing between saturated or unsaturated types. Unsaturated fatty acids have double bonds between their carbon molecules, while the unsaturated type does not have these bonds(19). Unsaturated fatty acids are chemically more unstable and may be of the monounsaturated type, when they have only one double bond, or polyunsaturated when they have two or more double bonds(20).

Unsaturated fatty acids may present a cis or trans configuration. They are characterized as a cis fatty acid when the hydrogen molecules in their geometric configuration are presented in the same plane as that of the double carbon bond. They are characterized as an unsaturated fatty acid of the trans type when their hydrogen molecules are on the opposite side of the double carbon bond. The trans fatty acid is a geometric isomer of the original *cis* fatty acid; that is, it presents the same quantity of carbon, oxygen and hydrogen molecules but with a different spatial configuration(21), figure 1.

The two types of fatty acids may be found in nature, however, the cis configuration is more common, because the enzymes that synthesize fatty acids have a preference for this

configuration. The trans fatty acids found in nature are present, in a reduced manner, in meats and milk. More expressive incorporation of trans acids into the human diet occurred with the process of hydrogenation of vegetable oils, especially by the food industry. Heating vegetable oil also induces the formation of geometric isomers of polyunsaturated fatty acids, and so does the irradiation of foods(22).

Trans fatty acids are more stable than the cis fatty acids, and therefore less energetic. Because the cis isomers are more energetic, they would be involved in the synthesis of different cellular lipids. The change in the structure of fatty acids to the trans form modifies the melting point, increases the plasticity and oxidative stability of these fats. Elaidic acid, for example, (9trans-18:1) presents a melting point of 44^0C whereas, oleic acid (9cis-18:1) has a melting point of 13^0C (23).

Melting point is an important characteristic of fatty acids. The higher the melting point, the greater the quantity of thermal energy necessary to break down their molecular arrangements. This allows greater impregnation into the tissues such as the vascular endothelium, and particularly in the adipose tissue. As the carbonated chain length increases, the melting point also increases. However, the presence of double bonds makes the melting point fall. Due to the geometric configuration, the melting point of trans fatty acids is also higher(24).

When trans fatty acids are ingested and absorbed, they may change the composition and biochemical activity of the cell membranes, thus changing the physical properties of the membrane, and finally change its functions(25-26). The composition of phospholipids in the plasma membrane has a crucial influence on cellular growth and metabolic activity. In the last two decades, studies have suggested that the lipid composition in the diet influences the fatty acid profile of serum and the lipid content of the plasma membrane. In fact, the length of the fatty acid chains and the degree of saturation or unsaturation have been shown to change the fluidity and activity of various proteins associated with the membrane(27).

With the change in the physiological processes as a consequence of the incorporation of these fatty acids into the different tissues, evidence of different adverse clinical situations have been shown in the literature. Among these, for example, increase in triglycerides, change in lipoproteins such as increase in LDL, VLDL, reduction in HDL concentration(28), increase in insulin resistance, increasing the risk of diabetes Mellitus(29), increase in the production of pro-thrombotic factors, increase in reactive oxygen species(30), increased risk for CAD, especially Acute Myocardial Infarction(31), among other pathologies(32).

Studies such as that of Chajés and colls, have suggested that high ingestion of

industrial trans fatty acids could cause an increase in body weight, especially in women. Furthermore, as mechanism for the prevention of obesity, they have suggested limitation of the consumption of highly processed foods must be considered, as they are the main source of industrially produced trans fatty acids(33-34).

On the other hand, unsaturated fatty acids with cis configuration have been related to favorable effects on the metabolism. Evidence has been shown of association between the consumption of unsaturated fat and aspects such as reduction in: blood viscosity and plasma triglycerides; higher level of endothelium relaxation(35); improvement in insulin sensitivity(36), among others.

Evidence has been shown that the intermediate products of fatty acid metabolism are important for myoblast survival, proliferation, differentiation and fusion. Studies have suggested that the lipid metabolites derived from polyunsaturated fatty acids accelerated protein synthesis, and the fusion and growth of muscle cells in different animal models(37).

Fostok and colls, have demonstrated that oleic acid (cis, unsaturated fatty acid) supplementation attenuated incomplete repair actions, optimizing the regenerative capacity and contractile function of the injured muscle(38).

In spite of the body of evidence presented up to now, substitution of saturated fats with polyunsaturated fats in diets as a way of preventing cardiovascular diseases continues to be questionable. According to Hamley, in a meta-analysis published in 2017, the evidences available in the randomized clinical trials up to now suggested that the substitution of saturated fatty acids with polyunsaturated fatty acids(n-6) in diets was not sufficient to reduce the events of cardiovascular diseases, mortality due to coronary disease or total mortality. Furthermore, he suggested that his results have implications for present dietary counsel, in which the recommendations to reduce saturated fat and/or substitute saturated fatty acids with polyunsaturated fatty acids must not be emphasized, because the maintenance of these recommendations would probably not have the intended effect and could reduce the efforts towards (encouraging) persons to adopt other lifestyle changes that would most probably be more beneficial(39).

The chart below presents the summary of the studies cited above, on the effect of fatty acids on the cardiovascular and metabolic systems. Table I

FATTY ACID ABSORPTION AND METABOLISM

Approximately 90% of the lipids consumed are in the form of triglycerides(TG). The remaining 10% are in the form of cholesterol, cholesterol esters, phospholipids and free fatty

acids. Lipid digestion begins in the mouth and stomach with the action of the lingual and gastric lipases. They degrade the TGs into medium and short chain fatty acids. After the action of the lipases, they undergo the action of the biliary salts and substances produced by the pancreas such as pancreatic lipase that will degrade the TGs formed by long chain fatty acids(40).

In sequence, the short and medium chain free fatty acids are directly absorbed by the enterocytes of the intestinal mucosa, and are released into the venous blood stream. They are then transported to the liver by albumin, by the hepatic port circulation, or to the peripheral tissues in which they are directly absorbed and used as energy substrate. Whereas, the long chain free fatty acids, non-esterified cholesterol, phospholipids together with their biliary salts and the liposoluble vitamins (A, D E and K), form the mycelia, which are hydrophilic particles that facilitate lipid transport, and these liposoluble vitamins, through the membrane of the enterocytes(41).

After crossing the intestinal mucosa, the compounds of the mycelia, together with Apolipoprotein B-48, will form the primogenitor lipoprotein–Chylomicra or Chylomicrons. At this point, the fatty acids are again resynthesized into triglycerides and the free cholesterol is esterified. Therefore, the chylomicras mainly transport triglycerides and esterified cholesterol(42).

The chylomicras are then transported to the peripheral tissues, to which mainly fatty acids are released for energy production. To enable them to be released, these fatty acids must be cleaved from the glycol. Lipoprotein lipase is the enzyme that cleaves the triglycerides coming from the chylomicras and later from the VLDL. The free fatty acids are then absorbed by the adipose or muscle tissue, or then transported to other tissues by albumin (40).

Lypolysis comprises four stages: cleavage of the triglycerides in the blood; beta-oxidation of the fatty acids; the citric acid cycle, and the electron transport chain. The first stage, as previously described, occurs in the blood, where the triglycerides present in the low density lipoproteins are cleaved by lipoprotein lipase. In this process, the fatty acids are released from the glycerol and transported up to the sarcolema (plasma membrane of the muscle cell) by albumin. On entering the cell, the second stage - beta-oxidation - occurs. In this stage, the fatty acids undergo the action of enzymes, such as thiokinase, present in the external membrane of the mitochondria. The mitochondria have two membranes - internal and external - and a space between these membranes. Beta-oxidation is finalized in the external membrane of the mitochondria, the site where thiokinase is found, which will finalize this process However, the long chain fatty acids, differently from the medium and short chain

types, are not permeable to the internal membrane of the mitochonndria, and require the action of carnitine, nutrient transporter of AcetylCoA, resulting from the catabolism of the long chain fatty acids through the internal membrane of the mitochondria(43).

Oxidative metabolism allows energy to be obtained from fatty acids in an intramitochondrial localization. Thus, to enable acyl-CoA to be used by it (oxidative metabolism), it is necessary to overcome the impermeability of the external and cytoplasmic membrane of the mitochondria to attain the acyl-CoA. The enzyme responsible for this transport is Carnitine-CoA acyltransferase (Carnitine O-Palmitoyltransferase). This enzyme presents greater specificity for Palmitoyl-CoA, however, it catalyzes transport of fatty acids with a carbonated chain length between C4 and C18. Fatty acid chains longer than these are more difficult to be transported. Once inside the mitochondria, acyl-CoA may be used in the lipolytic metabolism of LYNEN(44).

Carnitine palmityl transferase was historically seen as the only regulator of fatty acid oxidation. However, other FA translocators, such as FAT/CD36 have been identified. Specifically, FAT/CD36 appears to have a differentiated mechanism of action with respect to fatty acid oxidation during exercise, influencing lipid transport through the sarcolemal membrane and to the mitochondria(45).

Differently from the striated muscles, adipose tissue allows the entry and exit pathway of fatty acids. While the fatty acids only enter into the muscles with the purpose of producing energy, whereas, on entering into the adipose tissue, the fatty acids may produce energy and may also be accumulated. When necessary, the fatty acids accumulated in the form of triglycerides may be hydrolyzed and thus release fatty acids into the blood stream, and these are transported by albumin to be used in other tissues for the production of energy. The main consumer of these fatty acids released by the adipose tissue are the striated cardiac and skeletal muscles(43).

FATTY ACID METABOLISM DURING EXERCISE

The process of fatty acid uptake and oxidation is important for ATP resynthesis (46) (46). Once the fatty acids enter into the skeletal fiber, they have different destinations, depending on the metabolic state of the cells. In conditions of rest, the plasma fatty acids are conducted to triglyceride synthesis as the first destination, instead of being moved to the mitochondria for oxidation(46).

As the exercise progresses, long chain fatty acids, provided by the blood or from hydrolysis of intramuscular triacylglycerols, are metabolized to generate energy. The supply

of fatty acids from the hydrolysis of intramuscular triacylglycerols is limited and during exercise; the myocytes consume approximately 90% of the free fatty acids derived from blood plasma(44).

Small quantities of the triglycerides are stored within the lipid droplets in the skeletal muscle, and may be hydrolyzed to produce fatty acids for energy production by means of β oxidation and oxidative phophorylation. Although there has been some controversy about the quantitative importance of intramyocellular(IMTG) as metabolic substrate, recent studies have demonstrated a substantial contribution by IMTG to energy production(47).

There are three lipases expressed in the skeletal muscle, which are responsible for the degradation of TG: monoacylglycerol lipase; Adipose triglyceride lipase(ATGL) and hormone-sensitive lipase(HSL).

ATGL is the first step of TG lipolysis in the skeletal muscle of humans and mice, resulting in the release of one fatty acid molecule. Monoacylglycerol lipase is responsible for the hydrolysis of monoacylglycerol, releasing glycerol and fatty acids. The HSL catalyzes the hydrolysis of TG to release FA in the cytoplasm(43). The HSL is highly present in the type I oxidative fibers of skeletal muscle and is activated by adrenergic stimulation and contraction. ATGL is activated by the comparative identification of genes-58(CGI-58), These proteins are localized on the surface of the mitochondria, and are preferentially expressed in oxidative muscle, such as the cardiac and soleus muscles(48). Resistance training leads to increased levels of ATGL, increasing intramuscular lypolysis, particularly in Type I oxidative fibers(49). Studies such as that of Roepstorff et al have demonstrated that exercise triggered the rapid activation of HSL dependent on protein kinases in human beings, promoting the release of FA(50).

The mechanisms that regulate exercise-induced lipolysis in the skeletal muscle have not yet been completely elucidated, and may be more complex than lipolysis in adipose tissue(51).

Studies have also suggested that physical exercise performed in an acute manner promotes changes in the fatty acid transport genes, preceding increases in RNAm expression(52). This will allow greater metabolization of fatty acids, although the latter possibility has not yet been tested(53).

According to the research of Kim and colls, physical exercise is also capable of significantly increasing the expression of components of the metabolic pathway and components related to the redox signal induced a similar increase in the FAT / CD36 content of the cell membrane of skeletal muscle in rats(54).

In a randomized clinical trial published in 2017, the response of post-prandial triglycerides was observed to be attenuated by low to moderate intensity periodized exercises, when measured after 24 hours(55).

The use of lipids is modulated by the availability of fatty acids in the plasma. In obese subjects, the plasma AG levels are more elevated. Glycolytic activity is altered in this population, and lipid metabolism may be a preferential route. In obese subjects, the metabolic responses of fatty acid mobilization appear to be less favored by aerobic activity, however, the responses are not yet conclusive(56).

Recent studies have reinforced the differences in the patterns of lipolysis stimulation between thin and obese subjects during physical exercise. The difference in the lipolytic rate appear to be due to differences in the quantity or activity of the lipases present in the skeletal muscle, especially ATGL, and not the mRNA levels(57). Table II.

FATTY ACID CONSUMPTION, INFLAMMATORY AND ENDOTHELIAL DYSFUNCTION STATUS

There are various mechanisms by means of which diet increases or diminishes the risk for Cardiovascular Diseases. Investigation of the mechanisms that determine atherosclerosis have suggested that an inflammatory process plays a central role in its development, progression and outcomes. This inflammatory process causes structural and functional changes in the blood vessel walls, which leads to endothelial dysfunction and the development of atherosclerotic lesions(58). Calorie-restriction diets are known to reduce the circulating levels of C-reactive protein, which is a systemic inflammation marker that may also play its own role in the inflammatory process, and many studies have shown that it prevented cardiovascular events(59). Diets rich in omega-3 fatty acids appear to reduce atherosclerosis by means of the process of down-regulation of intracellular mechanisms that lead to the expression of pro-atherogenic genes(60).

The vascular endothelium is considered a dynamic tissue, an “organ” controlling important functions, such as coagulation, maintenance of blood circulation, vagal tonus, fluidification and inflammatory responses. Among these various functions, the endothelium is also responsible for the production of vasodilator and vasoconstrictive substances. Nitric oxide is the main factor in dilator responses and is directly involved in endothelial dysfunction(61).

The term “endothelial dysfunction” refers to an imbalance in endothelial production of mediators that regulate vascular tonus, platelet aggregation, coagulation and fibrinolysis.

Endothelial dysfunction is also frequently reported as worsening in endothelium-dependent relaxation, caused by loss of nitric acid(NO) bioavailability, although changes in other vasoactive substances have also been found(62).

Nitric Oxide has diverse antiatherogenic functions, among them, inhibition of smooth muscle cell production, inhibition of platelet aggregation and antioxidant properties. Its release is stimulated by the shear force exerted on the endothelium by blood; this fact is shown by the higher level of NO released from the arteries, in comparison with veins(61).

In the post-prandial state, a longer period of time during which triglyceride-rich(TG) lipoprotein levels remain elevated, may lead to endothelial dysfunction. This results in increase in the inflammatory response, lower level of nitric acid availability, and increase in oxidative stress - changes involved in the genesis of atherosclerosis(63).

After a meal with a high fat content, healthy individuals present a significant increase in the concentrations of proinflammatory cytokines, TNF-α, IL-6 and adhesion molecules (intercellular adhesion molecule-1 - ICAM-1 and Vascular cellular adhesion molecule-1 - VCAM-1), when compared with a meal with a high carbohydrate content. These changes may also be prevented with the use of Vitamin E, suggesting that oxidative stress regulates the increase in cytokines and adhesion molecules. Studies have demonstrated that the post-prandial triglyceride levels, and not those coming from adipocytes in fasting are more sensitive markers of atherosclerosis(64-65).

Elevation of C Reactive Protein(CRP) begins around 6 hours after inflammatory stimulation; it has a half-life of approximately 19 h, and its maximum value is attained in 24-72 hours. Its plasma concentration is constantly low, and does not present circadian variations, in contrast with some coagulation proteins and others of the acute inflammatory stage. Once stimulation has been concluded, the values return to normal after 7 days.

EXERCISE, INFLAMMATORY RESPONSE AND ENDOTHELIAL DYSFUNCTION

Although studies have shown evidence that the practice of physical activity prevents the genesis and progression of atherosclerotic disease, the mechanisms to explain this effect have not yet been completely elucidated. Ghisi and colls(61) suggested that the factor responsible for this effect is related to the change in vascular tonus and endothelial function.

Atherosclerosis development and progression partly depend on the migration of monocytes to the blood vessels, to become active and begin the release of cytokines. According to Teodoro and colls(66) the first cytokines in the cascade are the tumor necrosis

factor (TNF-α) and interleukin1 (IL1) considered proinflammatory cytokines. After an acute exercise session, there is no increase in the proinflammatory cytokines, suggesting that physical activity suppresses the entry of these cytokines into the plasma.

Paton and colls(67), after a study with healthy and sedentary subjects, concluded that exercise performed at 50% to 70% intensity continuously for 6 months was capable of improving the inflammatory response, coagulation and fibrinolytic potentials, reducing the risk for cardiovascular disease.

The effects of physical exercise on endothelial function, has been demonstrated in animal and human experiments, however, the literature is still controversial relative to the intensity of effort necessary to cause protective effects. The intensity most tested in both humans and animals is the moderate level, however, there are some evidences that high intensity, acute aerobic exercise increased the chance of cardiovascular events, but when performed chronically, it was associated with decreased occurrence of these events and mortality (63).

MacEneaney and colls(68) in a study about the effect of post-prandial lipemia on the inflammatory markers and endothelial activation in adolescents, found that physical exercise did not change the values of C-Reactive protein, TNF-α, IL-6 and adherence molecules in circulation, showing that in spite of significant reductions in hyperlipemia, exercise did not change the inflammatory response in 6h of observation. The findings of this study corroborated those of Dekker and colls(69) in which physical exercise did not significantly change the IL-6 values, although a trend towards reduction was perceived when compared with the control group.

In the study of Tyldum and colls(70) when they studied vasodilation mediated by flow after lipid overload, with and without physical exercise, showed evidence that with high intensity, periodized physical activity, the vessel diameter increased when compared with that of the control, demonstrating vasodilation secondary to physical activity.

Physical exercise has been associated with an increase in the nitric oxide synthase enzyme, with an influence on the increase in nitric oxide, providing a protective effect against endothelial dysfunction through physical exercise. Physical exercise also induces the release of extracellular superoxide dismutase which, according to Vourina(66), is an enzyme that acts in the antioxidant process.

Studies have shown evidence that dietary supplementation with polyunsaturated fatty acids acted on reducing inflammatory response(71). The beneficial effects of dietary supplementation with polyunsaturated fatty acids on exercise performance and on oxidative

balance of physical activity have also been shown in other studies(72) although the effects associated with intense physical activity on the immune response are still contradictory(73).

According to Capó and colls, exercise increased the activated levels of the anti-inflammatory response, increasing anti-inflammatory gene expression after the exercise, particularly in the group of young individuals(74). Recent studies have reinforced the idea that physical exercise attenuates the inflammatory response(75).

FATTY ACIDS AND OXIDATIVE STRESS

Oxidative stress formation, according to Ghisi(61) would be one of the main factors responsible for triggering atherogenesis, and that the superoxide anion(O_2^-) and oxidated LDL would be the main free radicals involved in this process. In addition to this, Reactive Oxygen Species(ROS) could interact with NO and form the peroxynitrite anion($ONOO^-$) and nitrogen dioxide(NO_2), which would be responsible for potentializing the inflammatory lesion, favoring the progression of atherosclerosis (66).

Lipid overload induces an increase in triglyceride-rich lipoprotein - TRLp, reduction in HDL and hyperinsulinemia. This metabolic condition leads to the formation of free radicals, which are reduced, according to the antioxidant capacity (endogenous and/or exogenous) present, determining oxidative stress. The free radicals stimulate tissues to secrete cytokines (TNF-α, IL-1 and IL-6), probably through the macrophages, thus stimulating the formation of adhesion molecules. The generation of reactive oxygen species diminishes the bioavailability of free NO, resulting in a lower level of endothelium-dependent vasodilation, and also in the formation of peroxynitrite($ONOO^-$), a potent and long lasting oxidant. These processes are associated with the genesis and progression of atherosclerotic lesions (76).

In the human body, there are lines of defense against the atherosclerotic process, among them the antioxidant enzymes nitric oxide(NO) and endothelial nitric oxide synthetase(eNOS); superoxide dismutase(SOD), catalase(CAT) and glutathione peroxidase(GPX). NO is an important factor responsible for the relaxation of arterial vessels: depending on the medium in which this may function as an oxidant or reducer, its oxidation produces nitrites and nitrates. Its anti-atherosclerotic functions include: inhibition of adhesion and migration of leukocytes, impeding platelet aggregation, reduction in endothelial permeability to lipoprotein macromolecules, impeding the sub-endothelial accumulation of LDLc and its oxidation, among others. These factors, among others, are related to modulation of the inflammatory response (76).

According to the data from the study of Tyldum(70) the effects of a diet rich in fats on reducing the antioxidant capacity appear, on an average, 30 minutes after ingestion.

EXERCISE AND OXIDATIVE STRESS

Light to moderate physical exercise performed regularly is recommended for the maintenance of health and prevention of innumerable diseases It also reduces the production of oxidants and occurrence of oxidative damage; improves the antioxidant defense system; increases the resistance of organs and tissues against the harmful action of the ROS, and diminishes systemic inflammation (77). However, there is a body of evidence suggesting that physical exercise, particularly the more intense types, are associated with both muscular damage and elevated ROS production (78).

In the study of Wang et al, when they tested different intensities of physical exercise in sedentary individuals with 40% VO_2 max, with 60% VO_2 max and with 80% VO_2 max for 40 min, they verified that in the acute form, the highest intensity resulted in higher production of oxidated LDL. This caused an increase in the reactive oxygen species in the monocytes, when compared with the light and moderate intensities. They concluded that in their study, acute, high intensity physical exercise caused greater oxidative stress in sedentary individuals (79).

In the study of Tyldum et al(69) about the effect of acute exercise of different intensities on the antioxidant capacity, they showed that both moderate and high intensity exercises interfered positively in the reduction of antioxidant capacity influenced by diet, however, the results were more expressive for the high intensity exercises.

According to Tyldum et al(69) exercise and antioxidant function revealed an interesting paradox; in the acute form, an increase occurs in the levels of free radicals in the blood and muscle, which may be responsible for the inactivity of large quantities of nitric oxide and negatively change the endothelial impact mediated by vasodilation. However, the results of the study revealed an increase in the antioxidant capacity in the acute stage of exercise. The authors suggested that during the acute stage of the exercise, a transfer of antioxidants occurs between the muscle and vasculature, favoring the balance towards an inclination to favor the antioxidant effect, providing a similar effect to that offered by a diet containing antioxidants.

Sureda et al, showed evidence that acute physical exercise performed at high intensities, induced oxidative damage in the blood cells such as erythrocytes and lymphocytes, but not in the neutrophils (78).

Habitual low to moderate intensity exercise is responsible for an increased cellular antioxidant defense system; reduction in lipid peroxidation, and protective effect against diseases associated with chronic inflammation (77).

Some studies have pointed out that intense exercises are associated with an increase in free radical formation. In other studies, acute and intense exercises presented significant and rapid responses in endothelial function. However, the intensity of exercise necessary to cause antioxidant responses is still (a) controversial (topic) in the literature.

Below, please find four summaries showing the articles presented in this section, with reference to the effect of physical exercise on the inflammatory response, endothelial dysfunction and oxidative stress. Table III.

CONCLUSION

Fatty acids, depending on their biochemical characteristics and spatial configuration, have differentiated effect on cardiovascular health, however, studies still present contradictory results about the therapeutic use of certain fatty acids. Physical activity appears to benefit fatty acid metabolism and attenuate the complications secondary to the consumption of certain fatty acids, and potentializes the positive effects of distinct fatty acids. However, variants of physical activity, such as intensity, duration, time of observation of effects of the results, limit the authors to concluding, with a certain degree of certainty, about the effect of physical exercise on fatty acids and secondary complications, since the studies in the literature continue to be contradictory. The authors perceived the need for further consistent studies that emphasize (the effects of) physical activity, with its variations, on the different types of fatty acids, and on their secondary complications.

REFERENCES

1- Moran AE, Forouzanfar MH, Roth GA, Mensah GA, Ezzati M, Murray CJ, et al. Temporal trends in ischemic heart disease mortality in 21 world regions, 1980 to 2010: the Global Burden of Disease 2010 study, Circulation. 2014;129:1483e1492.
2- Finucane MM, Stevens GA, Cowan MJ, Danaei G, Lin JK, Paciorek CJ, et al. National, regional, and global trends in body-mass index since 1980: systematic analysis of health examination surveys and epidemiological studies with 960 country-years and 9.1 million participants, Lancet, 2011; 377: 557e567.
3- Joint ESC. Guidelines / Atherosclerosis. 2016; 252: 207e274.
4- Gottlieb MG, Bonardi G, Moriguchi EH. Fisiopatologia e aspectos inflamatórios da aterosclerose. *Scientia Medica*, 2005; *15*(3):203-207.

5- Shivappa, N, Blair CK, Prizment AE, Jacobs DR, Steck SE, Hébert JR. Association between inflammatory potential of diet and mortality in the Iowa Women's Health study. *European Journal of Nutrition*, 2016; *55*(4): 1491–1502. http://doi.org/10.1007/s00394-015-0967-.
6- Martin CA; Matshushita M, Souza NE. Ácidos graxos trans: implicações nutricionais e fontes na dieta. *Rev. Nutr.* [online]. 2004; 17(3): 351-359.
7- Costa AGV, Bressan J, Sabarense CM. Acidos graxos trans: Alimentos e efeitos na saúde. Archivos latinoamericanos de nutricion (ALAN), 2006; 56(1): 1-9.
8- Oomen CM, Ocke MC, Feskens EJ, Van Erp-Baart MA, Kok FJ, Kromhout D. Association between trans fatty acid intake and 10-year risk of coronary heart disease in the Zutphen Elderly Study: a prospective population-based study. Lancet. 2001; 357(1): 746-571.
9- Perez-Matos MC, Morales-Alvarez MC, Mendivil CO, Lipids: A Suitable Therapeutic Target in Diabetic Neuropathy?. J Diabetes Res. 2017; 2017:6943851. doi:10.1155/2017/6943851. Epub 2017 Jan 16.
10- Lopez-Garcia E, Schulze MB, Meigs JB, Manson JE, Rifai N, Stampfer MJ, et al. Consumption of trans fatty acids is related to plasma biomarkers of inflammation and endothelial dysfunction. J Nutr. 2005; 135(1): 562-6.
11- Jabbour G, Iancu H-D, Paulin A, Lavoie J- M, Lemoine-Morel S, Zouhal H. Effects of Acute Supramaximal Cycle Exercise on Plasma FFA Concentration in Obese Adolescent Boys. PLoS ONE, 2015; 10(6): e0129654.
12- Bilet L, Brouwers B, van Ewijk PA, Hesselink MK, Kooi ME, Schrauwen P, et al. Acute exercise does not decrease liver fat in men with overweight or NAFLD**.** Sci Rep. 2015;13(5):9709. doi:10.1038/srep09709.
13- Thivel D, Metz L, Julien A, Morio B, Duche P. Obese but not lean adolescents spontaneously decrease energy intake after intensive exercise. Physiol Behav, 2014; 123: 41–46.
14- Bodel NG, Gillum T. 90 Minutes of Moderate-Intensity Exercise does not Attenuate Postprandial Triglycerides in Older Adults. Int J Exerc Sci. 2016; 9(5): 677-684.
15- Davitt PM, Arent SM, Tuazon MA, Golem DL, Henderson GC. Postprandial triglyceride and free fatty acid metabolism in obese women after either endurance or resistance exercise. J Appl Physiol. 2013; 114:1743–54.
16- Durstine JL, Thompson PD. Exercise in the treatment of lipid disorders. Cardiol Clin 2001;19:471-88.
17- Rodrigues LEA. Lipídios: aspectos bioquímicos e médicos. EDUFBA, Salvador-Bahia, 2006.
18- Mäkinen S, Nguyen HY, Skrobuk P, Koistinen HA. Palmitate and oleate exert differential effects on insulin signalling and glucose uptake in human skeletal muscle cells. Endocr Connect. 2017; 5:17-0039. doi: 10.1530/EC-17-0039
19- Kadhum AA, Shamma MN. Edible lipids modification processes: A review Crit Rev Food Sci Nutr. 2017; 57(1):48-58.
20- Moreira NX; Curi R, Mancini Filho J. Ácidos graxos: uma revisão. Nutrire; rev. Soc. Bras. Alim. Nutr. 2002; 24:105-123.
21- Kadhum AA, Shamma MN. Edible lipids modification processes: A review Crit Rev Food Sci Nutr. 2017; 57(1):48-58.
22- BRito MS; Villavicencio, ALCH, Mancini-Filho J. Effects of irradiation on trans fatty acids formation in ground beef. Radiat. Phys. Chem., Oxford, 2002; 63:337-340.
23- Aued-Pimentel S. et al. Ácidos graxos saturados versus ácidos graxos trans em biscoitos. Rev. Inst. Adolfo Lutz, 2003; 62(2):131-137.
24- Kadhum AA, Shamma MN. Edible lipids modification processes: A review Crit Rev Food Sci Nutr. 2017; 57(1):48-58.
25- Moreira NX; Curi R, Mancini Filho J. Ácidos graxos: uma revisão. Nutrire; rev. Soc. Bras. Alim. Nutr., 2002; 24:105-123.
26- Sugano M, Ikeda I. Metabolic interactions between essential and trans fatty acids. Curr. Opin. Lipidol, 1996; 7:38- 42.
27- Abreu P, Leal- Cardoso JH, Ceccatto VM, Hirabara SM. Regulation of muscle plasticity and trophism by fatty acids: A short review. Rev. assoc mEd Bras 2017; 63(2):148-155.
28- Sandres TA, Oakley FR, Crook D, Coper JA, Miller GJ. High intakes of trans monounsaturated fatty acids taken for 2 weeks do not influence procoagulant and fibrinolytic risk markers for CHD in young healthy men. Br J Nutr. 2003; 89(6):767-76.
29- Perez-Matos MC, Morales-Alvarez MC, Mendivil CO, Lipids: A Suitable Therapeutic Target in Diabetic Neuropathy?. J Diabetes Res. 2017. doi: 10.1155/2017/6943851. Epub 2017 Jan 16
30- Zapolska-Downar D, Kosmider A, Naruszewicz M. Trans fatty acids induce apoptosis in human endothelial cells. J Physiol Pharmacol. 2005; 56(4):611-25.

31- Sun Q, Ma J, Campos H, Hankinson SE, Manson JE, Stampfer MJ, et al. A prospective study of trans fatty acids in erythrocytes and risk of coronary heart disease. Circulation. 2007;115(14):1858-65

32- Aued-Pimentel S. et al. Ácidos graxos saturados versus ácidos graxos trans em biscoitos. **Rev. Inst.** Adolfo Lutz, 62(2): 131 - 137, 2003.

33- Chajès V, Biessy C, Ferrari P, Romieu I, Freisling H, Huybrechts I, et al. Plasma Elaidic Acid Level as Biomarker of Industrial *Trans* Fatty Acids and Risk of Weight Change: Report from the EPIC Study. *PLoS ONE*, 2015;*10*(2), e0118206.

34- Ford, PA. et al. *Trans* fatty acid intake is related to emotional affect in the Adventist Health Study-2. Nutrition Research, 2016; 36 (6):509 – 517.

35- Sposito, AC. et al. IV Diretriz Brasileira sobre Dislipidemias e Prevenção da Aterosclerose: Departamento de Aterosclerose da Sociedade Brasileira de Cardiologia. *Arq. Bras. Cardiol.* [online]. 2007; 88(1)1: 2-19.

36- Abbott SK, Else PL, Hulbert AJ. Membrane fatty acid composition of rat skeletal muscle is most responsive to the balance of dietary n-3 and n-6 PUFA. Br J Nutr. 2010; 103(4):522-9.

37- Veliça PP, Khanim FL, Bunce CM. Prostaglandin D2 inhibits C2C12 myogenesis. Mol Cell Endocrinol. 2010; 319(1-2):71-8.

38- Fostok SF, Ezzeddine RA, Homaidan FR, et al. Interleukin-6 and Cyclooxygenase-2 downregulation by fatty-acid fractions of *Ranunculus constantinopolitanus*. *BMC Complementary and Alternative Medicine*. 2009; 9:44.

39- Hamley, S. The effect of replacing saturated fat with mostly n-6 polyunsaturated fat on coronary heart disease: a meta-analysis of randomised controlled trials. *Nutrition Journal*, 2017; *16*(30).

40- Kazantzis, M, Stahl A. Fatty Acid transport Proteins, implications in physiology and disease. *Biochimica et Biophysica Acta*, 2012. *1821*(5), 852–857.

41- Aon MA, Bhatt N, Cortassa SC. Mitochondrial and cellular mechanisms for managing lipid excess. *Frontiers in Physiology*, 2014; *5*(282).

42- Sztalryd C, Kimmel AR. Perilipins: lipid droplet coat proteins adapted for tissue-specific energy storage and utilization, and lipid cytoprotection. Biochimie. 2014; 96: 96-101.

43- Morales PE, Bucarey JL, Espinosa A. Muscle Lipid Metabolism: Role of Lipid Droplets and Perilipins. *Journal of Diabetes Research*, *2017*, 1789395.

44- Ewa H, Agnieszka K, Tomasz S, Adrian C. The role of fatty-acid transport proteins (FAT/CD36, FABPpm, FATP) in lipid metabolism in skeletal muscles. Postepy Hig Med Dosw. (online), 2008; 62: 433-441.

45- Smith BK, Bonen A, Holloway GP. A Dual Mechanism of Action for Skeletal Muscle FAT/CD36 During Exercise, Exercise and sport sciences reviews 2012; 40(4): 211-7.

46- Jayewardene AF, Mavros Y, Reeves A, Hancock DP, Gwinn T, Rooney KB. Interactions Between Fatty Acid Transport Proteins, Genes That Encode for Them, and Exercise: A Systematic Review. J Cell Physiol. 2016; 231(8):1671-87.

47- Watt MJ, Cheng Y. Triglyceride metabolism in exercising muscle. Biochim Biophys Acta. 2017; 23(17):30119-1.

48- Badin PM, Loubiere C, Coonen M, et al. Regulation of skeletal muscle lipolysis and oxidative metabolism by the co-lipase CGI-58. *Journal of Lipid Research*. 2012; 53(5):839–848.

49- Turnbull PC, Longo AB, Ramos SV, Roy BD, Ward WE, Peters SJ. Increases in skeletal muscle ATGL and its inhibitor G0S2 following 8 weeks of endurance training in metabolically different rat skeletal muscles. American Journal of Physiology. Regulatory, Integrative and Comparative Physiology. 2016; 310(2):R125–R133.

50- Roepstorff C, Vistisen B, Donsmark M, et al. Regulation of hormone-sensitive lipase activity and Ser563 and Ser565 phosphorylation in human skeletal muscle during exercise. The Journal of Physiology. 2004; 560(2): 551–562.

51- Casas M, Figueroa R, Jorquera G, Escobar M, Molgó J, Jaimovich E. IP(3)-dependent, post-tetanic calcium transients induced by electrostimulation of adult skeletal muscle fibers. *The Journal of General Physiology*. 2010;136(4):455–467.

52- Barres R, Yan J, Egan B, Treebak JT, Rasmussen M, Fritz T, et al. Acute exercise remodels promoter methylation in human skeletal muscle. Cell Metab, 2012; 15:405–411.

53- Egan B, Zierath JR. Exercise metabolism and the molecular regulation of skeletal muscle adaptation. Cell Metab, 2013; 17:162–184.

54- Kim J, Lee KP, Lee DW, Lim K. Piperine enhances carbohydrate/fat metabolism in skeletal muscle during acute exercise in mice. Nutrition & Metabolism. 2017;14:43.

55- Homer AR, Fenemor SP, Perry TL, Rehrer NJ, Cameron CM, Skeaff CM, et al. Regular activity breaks combined with physical activity improve postprandial plasma triglyceride, nonesterified fatty acid, and insulin responses in healthy, normal weight adults: A randomized crossover trial**.** J Clin Lipidol. 2017;

19(17): 30351-3.
56- Jabbour G, Iancu HD, Paulin A, Lavoie JM, Lemoine-Morel S, Zouhal H. Effects of Acute Supramaximal Cycle Exercise on Plasma FFA Concentration in Obese Adolescent Boys. PLoS ONE, 2015; 10(6).
57- Petridou A, Chatzinikolaou A, Avloniti A, Jamurtas A, Loules G, Papassotiriou I, et al. Increased Triacylglycerol Lipase Activity in Adipose Tissue of Lean and Obese Men During Endurance Exercise J Clin Endocrinol Metab. 2017;12.
58- Nojiri, S, Daida H. Atherosclerotic Cardiovascular Risk in Japan. *Japanese Clinical Medicine*, 2017; *8*.
59- Iso H, Cui R, Date C, et al. C-reactive protein levels and risk of mortality from cardiovascular disease in Japanese: the JACC Study. Atherosclerosis. 2009; 207:291–297.
60- Shivappa, N, Blair CK, Prizment AE, Jacobs DR, Steck SE, Hébert JR. Association between inflammatory potential of diet and mortality in the Iowa Women's Health study. European Journal of Nutrition, 2016; *55*(4), 1491–1502.
61- Ghisi GLM. et al. Exercício físico e disfunção endotelial. Arq Bras Cardiol, 2010; 95(5):130-7.
62- Connor M. et al. The Effect of acute aerobic exercise on pulse wave velocity and oxidative stress following postprandial hypertriglyceridemia in health men. Eur J. Appl Physiol, 2007; 100:225-234.
63- Stapleton PA, Goodwill AG, James ME. Hypercholesterolemia and microvascular dysfunction: interventional strategies. J. Inflamm (Lond). 2010; 18;7:54
64- Nappo F, Esposito K, Cioffi M, Giugliano G, Molinari AM, Paolisso G, et al. Postprandial endothelial activation in healthy subjects and in type 2 diabetic patients: role of fat and carbohydrate meals. J Am Coll Cardiol, 2002; 39:1145-50.
65- Pinho, RA. Araújo MC, Ghisi GLM, et al. Doença arterial coronariana, exercício físico e estresse oxidativo. Arq. Bras. Cardiol. 2010; 94(4).
66- Vuorimaa T, Ahotupa M, Irjala K, Vasankari T. Acute Prolonged Exercise Reduces Moderately Oxidized LDL in Healthy Men. Int J Sports Med, 2005;26:420-5.
67- Paton, C. M. et al. Hemostatic response to postprandial lipemia before and after exercise training. J. Appl. Physiol. 2006; 101:316-321.
68- MacEneaney JO, et al. effect of prior exercise on postprandial lipemia and markers of inflammation and endothelial activation in normal weighth and overweight adolescent boys**.** Eur J. appl physiol. 2009; 106:721-729.
69- Dekker MJ, Graham ET, Ooi TC, Robinson LE. Exercise prior to fat ingestion lowers fasting and postprandial VLDL and decreases adipose tissue IL-6 and GIP receptor mRNA in hypertriacylglycerolemic men. Journal of Nutritional Biochemistri, 2010; 21: 983-990.
70- Tyldum GA et al. Endothelial dysfunction induced by postprandial lipemia: complete protection afforded by high intensity aerobic interval exercise.J Am Coll Catdiol. 2009;53(2):200-2006.
71- Medina-Remon A, Tresserra-Rimbau A, Pons A., Tur J.A., Martorell M., Ros E, et al. Effects of total dietary polyphenols on plasma nitric oxide and blood pressure in a high cardiovascular risk cohort. The predimed randomized trial. Nutr. Metab. Cardiovasc. Dis. 2015;25:60–67.
72- Mickleborough T.D. Omega-3 polyunsaturated fatty acids in physical performance optimization. Int. J. Sport Nutr. Exerc. Metab. 2013;23:83–96.
73- Garcia JJ, Bote E, Hinchado MD, Ortega E. A single session of intense exercise improves the inflammatory response in healthy sedentary women. J. Physiol. Biochem. 2011;67:87–94.
74- Capó X, Martorell M, Sureda A, Riera J, Drobnic F, Tur, JA, Pons A. Effects of Almond- and Olive Oil-Based Docosahexaenoic- and Vitamin E-Enriched Beverage Dietary Supplementation on Inflammation Associated to Exercise and Age. *Nutrients*, 2016;*8*(10): 619.
75- Rocha-Rodrigues S, Rodríguez A, Gonçalves IO, Moreira A, Maciel E, Santos S, et al. Impact of physical exercise on visceral adipose tissue fatty acid profile and inflammation in response to a high-fat diet regimen.Int J Biochem Cell Biol. 2017; 87:114-124.
76- Pinho RA, Araújo MC, Ghisi GLM, et al. Doença arterial coronariana, exercício físico e estresse oxidativo. Arq. Bras. Cardiol. 2010, 94(4).
77- Capó X, Martorell M, Sureda A, Riera J, Drobnic F, Tur JA, Pons A. Effects of Almond- and Olive Oil-Based Docosahexaenoic- and Vitamin E-Enriched Beverage Dietary Supplementation on Inflammation Associated to Exercise and Age. *Nutrients*, 2016. *8*(10): 619.
78- Sureda A, Tauler P, Aguiló A, Cases N, Fuentespina E, Córdova A, et al. Relation between oxidative stress markers and antioxidant endogenous defences during exhaustive exercise.Free Radic Res. 2005; 39(12):1317-24.
79- Jong-Shyan W, Lee T, Chow S. Role of exercise intensities in oxidized low-density lipoprotein-mediated redox status of monocyte in men. Journal of Applied Physiology. 2006; 101(3):740-744.

Quadro I: Summary of the studies about fatty acids and effect on the cardiovascular and metabolic systems

AUTHORS	YEAR	TYPE OF STUDY	POPULATION	RESULTS
Sandres TA, Oakley FR, Crook D, Coper JA, Miller GJ	2003	A Randomized Clinical Trial	29 men	Trans diet reduced HDL cholesterol, however did not change the hemostatic risk factors
Zapolska-Downar D, Kośmider A, Naruszewicz M.	2005	Controlled Study	Umbilical cells	Increase in apoptosis and induction of intracellular production of Reactive Oxygen Species
Sun Q, Ma J, Campos H, Hankinson SE, Manson JE, Stampfer, MJ, et al	2007	Case Control Study	493 cases and controls	The highest total trans fatty acid content in the erythrocytes was associated with an elevated risk of Cardiovascular Disease.
Chajès V, Biessy C, Ferrari P, Romieu I,, Freisling H, Huybrechts I, Et al	2015	Prospective study	1949 participants	High consumption of trans fatty acids induced an increase in weight
Ford PA, Jaceldo, Siegl K, Lee JW, Tonstad S	2016	Cross-sectional Cohort Study	8771 participants	Lower ingestion of dietary trans fatty acids had beneficial effects on the emotional status
Abbott SK, Else PL, Hulbert AJ.	2010	Experimental Study	Young rats	Consumption of trans fatty acids interfered in the composition of fatty acid composition of phospholipids of the rat skeletal muscles.
Sabreen F Fostok Rima A, Ezzeddine, Fadia R Homaidan, Jamal A Al-Saghir, Ralph G Salloum, Najat A Saliba et al	2009	Experimental Study	Young rats	Monounsaturated fatty acids in the cis configuration increased the anti-inflammatory effects
Steven Hamley	2017	Meta-analysis	Studies of the Randomized Clinical Trial Type	The evidence available in adequately controlled randomized controlled trials suggested that the replacement of saturated fatty acids with the majority of the polyunsaturated fatty acids n-6 would be unlikely to reduce the events of Cardiovascular Disease, mortality due to coronary disease, or total mortality.
Briolay A, Jaafar R, Nemoz G, Bessueille L.	2013	Experimental Study	Rat skeletal muscle	Supplementation of monounsaturated fatty acids such as the oleic type, changed the membrane lipid composition

Quadro II: Effect of exercise on fatty acid metabolism

AUTHORS	YEAR	TYPE OF STUDY	POPULATION	RESULTS
Turnbull P. C., Longo A. B., Ramos S. V., et al	2016	Randomized Experimental Study	10 male rats per Control and Experimental Groups	Resistance training increased the TGLA, increasing intramuscular lypolysis, particularly in Type I oxidative fibers
Roepstorff C., Vistisen B., Donsmark M., et al	2004	Self-controlled Study	8 moderately trained men	Exercise triggered rapid LHS activation dependent on protein kinases, promoting AG release.
Barres R, Yan J, Egan B, Treebak JT, et al	2012	Self-controlled Study	14 sedentary and healthy participants	Acute physical exercise promoted change in fatty acid gene transporters.
Kim J, Lee K-P, Lee D-W, Lim K	2017	Experimental Study	18 Rats	Exercise induced significant increase in expression of components of the lipid metabolic pathway and components related to the redox signal.
Homer AR, Fenemor SP, Perry TL, et al	2017	A Randomized Clinical Trial	36 adult participants	The post-prandial triglyceride response was attenuated with physical exercise
Jabbour G, Iancu H-D, Paulin A, et al	2015	Comparative Study	45 obese adolescents	Greater lipid mobilization in adolescents with changed weight when compared with those with normal weight.
Petridou A, Chatzinikolaou A, Avloniti A, et al	2017	Comparative Study	16 adults divided into two groups 7 thin and 9 obese subjects	No change in the mRNA levels during exercise was found, but the obese presented lower levels of mRNA, ATGL and HLS, in comparison with the adults with normal weight.

Quadro III: Effect of physical exercise on inflammatory response, endothelial dysfunction and oxidative stress

AUTHORS	YEAR	TYPE OF STUDY	POPULATION	RESULTS
Paton C.M. et al	2006	Prospective study	Healthy participants	Improved inflammatory response, coagulation and fibrinolytic potentials, reduced risk of cardiovascular disease
MacEneaney, J. O. et al	2009	Comparative Study	18 adolescents divided into: 10 normal weight and 8 obese subjects	Moderate exercise before a meal with high fat content effectively reduced the post-prandial TG concentrations in adolescents with normal weight and changed weight, but did not reduce the concomitant post-prandial increase in white globules or IL-6.
Dekker, M. J, et al	2010	Self-controlled Study	9 obese men	Physical exercise did the significantly change the IL-6 values
Tyldum G. A. et al	2006	Self-controlled Study	8 healthy men	Acute exercise promoted a clinically protective effect on the vasculature that is dependent on the intensity of the exercise and strongly related to the antioxidant capacity induced by the exercise.
Vuorimaa. et al	2010	Self-controlled Study	10 healthy, trained men	Light intensity and long duration physical exercise acutely suppressed the oxidative stress loads, and was inversely related to the atherogenic process.
Garcia J.J., Bote E., Hinchado M.D., Ortega E.	2011	Systematic Review	10 articles	Although the responses to CRP were inconsistent, a single attack of exercise could increase both the activity and count of circulating IL-6, and the neutrophil count in non-trained adults.
Capó, X. et al	2016	Comparative Study	5 young adult athletes and 5 elderly athletes	Exercise increased the activated levels of the anti-inflammatory response, increasing anti-inflammatory gene expression after the exercise, particularly in the group of young individuals.
Rocha-Rodrigues S, et al	2017	Controlled Experimental Study	Rats divided into Control, Free Physical Activity and Resistance Exercise Groups	Exercise induced specific changes in fatty acids, irrespective of the composition of these in the diet, but only resistance exercise attenuated the inflammatory response.
Sureda A., et al	2005	Self-controlled Study	9 athletes	Exercise induced oxidative damage in the blood cells, such as erythrocytes and lymphocytes, but no in the neutrophils.
Jong-Shyan Wang, Tan Lee, Shu-Er Chow.	2006	Self-controlled Study	25 healthy, sedentary men	High intensity exercise increased the ROS production of monocytes induced by LDL, however, light and moderate exercise protected the individuals against the suppression of anti-oxidative capacity of the monocyte by LDL.

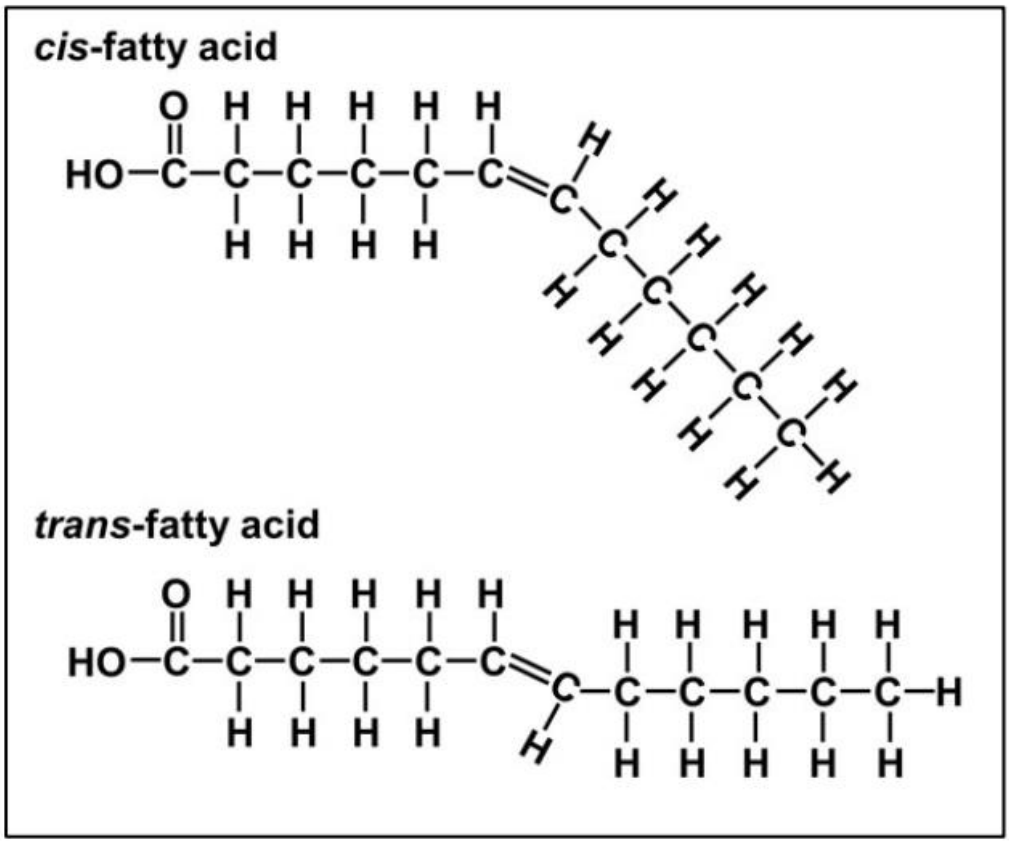


Figure 1: Illustration of a trans-unsaturated and a cis-unsaturated fatty acid.

3.2 Considerações sobre o metabolismo das gorduras durante o exercício fisico de baixa a moderada intensidade em mulheres obesas

A obesidade vem representando um importante problema de saúde pública uma vez que têm crescido a prevalência desta condição clínica. A preocupação com esta crescente refere-se, entre tantas outras, as complicações metabólicas atreladas a este estado. Dentre as mudanças metabólicas provocadas pela obesidade estão um estado basal alterado representado entre outros aspectos por um estado de hiperinsulinemia, aumento da resistência insulínica, aumento séricos de marcadores inflamatórios como adipocinas[31] e aumento dos níveis de ácidos graxos livres circulantes.

Reaven[32] foi um dos primeiros pesquisadores a perceber a relação do aumento de peso com a resistência insulínica e estado de hiperinsulinemia basal. Atualmente, a obesidade é considerada um fator de risco bem reconhecido para o desenvolvimento da resistência insulínica (RI).

Em indivíduos magros, as adipocinas, medeiam funções fisiológicas enquanto que em estados de doença metabólica, como na obesidade, as adipocinas têm efeitos alterados, modulando a resistência à insulina diretamente, afetando a via de sinalização da insulina ou indiretamente através da estimulação de vias inflamatórias[33].

As complicações referentes a este estado de hiperinsulinemia em repouso presente na obesidade relacionam-se entre outras a um menor metabolismo dos ácidos graxos no estado de repouso, refletindo em um aumento da concentração de ácidos graxos livres (AGLs). Altos níveis de AGLs podem interferir na utilização da glicose, através da diminuição da atividade da enzima glicogênio-sintase e aumento nos depósitos de triglicerídeos intramuscular[34].

Os efeitos do treinamento físico na ação da insulina e captação de glicose ainda carecem de esclarecimentos. Entretanto, Horowitz et al[35] sugerem que o exercício físico tenha condições de melhorar o metabolismo de lipídeos durante a atividade, devido ao aumento na oxidação dos ácidos graxos e a melhora da capacidade oxidativa da musculatura esquelética. Este aumento na oxidação lipídica parece ser um efeito importante do treinamento físico na melhora da sensibilidade insulínica em indivíduos obesos.

É sabido que a insulina exerce um efeito inibitório ao processo de lipólise, porém, durante o exercício físico, a concentração plasmática de insulina é reduzida, decorrente do efeito inibitório das catecolaminas sobre a liberação de insulina pancreática[36].

Os tipos de exercícios físicos que se beneficiam de forma significativa do metabolismo dos AG são aqueles de baixa intensidade, longa duração e com predominância

aeróbica, visto que há uma relação direta entre a intensidade do esforço e a utilização de glicose como substrato energético[37].

Sugere-se que em obesos exista uma limitação ao fornecimento de energia pela via de predominância anaeróbia uma vez que nesta população observa-se uma presença de fadiga prematura e uma a redução da ativação das fibras musculares do tipo II (fibras brancas). Neste contexto a atividade aeróbia encontra-se favorecida e o metabolismo dos ácidos graxos, passa a ser uma opção de fornecimento de energia mais viável[18]. Segundo Horowitz e Klein (2000)[38], durante a realização de exercícios de baixa intensidade a oxidação de AG parece ser derivada dos AG plasmáticos.

Quanto as diferenças do gênero no metabolismo dos lipídios, estudos como o de Davis et al[39] demonstraram que ao realizar exercícios de intensidade moderada, mulheres demonstram uma maior oxidação de lipídios e carboidratos em relação aos homens.

Em mulheres obesas que praticaram regularmente exercício físico de moderada intensidade, foi evidenciada menor concentração de citocinas pró-inflamatórias, aumento de adiponectina e moléculas de adesão vascular e aumento da resposta vascular. Os autores concluíram então que o exercício, acompanhado da perda de peso diminuiu o estado inflamatório e modulou a disfunção endotelial de mulheres obesas[40].

Um menor estado inflamatório estaria, entre outros aspectos, associado a uma maior expressão da adiponectina e por conseguinte uma maior sensibilidade insulínica nos tecidos, reduzindo no estado basal a insulina circulante. Desta forma, uma menor insulina circulante estaria associada à uma maior possibilidade de mobilizaçãoo de gorduras no estado pós-exercício, uma vez que a insulina aumentada inibe a mobilização de ácidos graxos favorecida pela inibição da enzima lipase lipoproteica. [31]

Em suma, algumas evidências na literatura sugerem que o metabolismo de ácidos graxos pode ser favorecido pela atividade física beneficiando especialmente a população feminina obesa. Porém a literatura ainda carece de resultados mais definitivos a partir de estudos com metodologisa mais consistentes.

4 MÉTODOS

Este estudo trata-se de um ensaio clínico randomizado, registrado no Clinical Trial sob o protocolo NCT03170973, com população acessível da Clínica Escola da Faculdade Adventista da Bahia, Cachoeira, BA, Brasil. As coletas foram realizadas durante o período de Setembro de 2015 a Maio de 2016.

Para a determinação do tamanho amostral foi considerada a regra de proporções, supondo-se que antes da intervenção 50% das mulheres teriam os valores de ácidos graxos acima da mediana e estimando que após a intervenção 15% permaneceriam com o nível de ácidos graxos acima da mediana, para alfa = 0,05 (bidirecional) e beta = 0,80, foram necessárias 33 mulheres em cada grupo.

Sessenta e seis voluntárias que apresentavam IMC acima de 24,9kg/m^2 foram selecionadas aleatoriamente e convidadas a participar do estudo. As voluntárias atenderam ao critério de inclusão do estudo que foram: idade entre 18 a 30 anos, IMC>24,9kg/m^2 e sedentarismo. Este último determinado com base no Questionário Internacional de Atividade Física-versão longa[29]. Foram excluídas mulheres que apresentassem doença cardiovascular, metabólica, hipotireoidismo, doenças renais parenquimatosas ou diabetes mellitus, histórico de alcoolismo ou tabagismo, uso de hipolipemiantes, corticóides, diuréticos, beta-bloqueadores e anticoncepcionais.

As mulheres foram divididas aleatoriamente, a partir de um sorteio, em dois grupos, exercício e controle, ambos com 33 voluntárias.

As voluntárias foram submetidas, após jejum de 12 horas, à coleta de sangue na veia antecubital para medição dos valores séricos basais de ácidos graxos, substâncias reativas ao ácido tiobárbiturico (TBARS), carbonilas, sulfidrilas, proteína-C reativa (PCR), triglicerídeos, colesterol total e frações, glicemia e insulina. A partir dos valores de glicemia e insulina foram calculados os valores do índice Homa-IR e Homa-Beta pela equação proposta por Mathews et al[30].

Passadas 12h da primeira coleta de sangue, as pacientes realizaram uma sessão de exercício físico em esteira ergométrica, dividida em 3 tempos: aquecimento, condicionamento e desaquecimento. O aquecimento foi de 7 minutos, o desaquecimento de 5 minutos e o tempo de condicionamento foi o correspondente ao gasto energético de 250Kcal[41]. O tempo médio em atividade física das participantes foi de 37±8 min. O controle da intensidade foi determinada pela velocidade. A inclinação da esteira foi mantida em 0^o durante toda a

atividade. A intensidade utilizada foi a leve, baseada na percepção de esforço de Borg[42], ou seja, na escala original correspondeu a um valor entre 9 e 11. Para um melhor entendimento dessa escala foi realizado um ancoramento prévio ao dia do exercício habituando as voluntárias a responderem de forma adequada quando solicitado sobre a intensidade do exercício. Foi utilizado cardiofrequencímetro que mediu o gasto energético com base na massa corporal, sexo e idade da voluntária.

Após a sessão de exercício físico elas foram orientadas a retornar para casa e manter a sua dieta habitual. Vinte e quatro horas após a primeira coleta de sangue, as voluntárias retornaram ao laboratório após um jejum de 12 horas e tiveram novamente amostras de sangue coletadas. Foram avaliadas as dietas dos dois dias anteriores ao exame de sangue através do recordatório alimentar de 24 horas.

As mulheres do grupo controle foram submetidas ao mesmo protocolo de coleta de dados do grupo experimental porém não realizaram o exercício 12h após a primeira coleta e foram orientadas a não realizarem exercício físico nos dois dias prévios a coleta de sangue.

Os pacientes foram avaliados quanto a dieta do dia anterior ao exame a partir de um recordatório alimentar de 24 horas. O recordatório de 24 horas, foi feito através de entrevista realizada no momento da coleta de sangue na qual os voluntários informaram o que haviam consumido no dia anterior à entrevista, nas três refeições principais e entre elas. O instrumento foi aplicado pelo mesmo examinador e para facilitar as respostas foram utilizadas medidas caseiras[43]. A avaliação quantitativa da dieta foi realizada com a utilização do software *Avanutri Revolution.* Para fins de análise considerou-se o consumo de macronutrientes, micronutrientes (vitaminas e minerais), colesterol, gorduras saturadas, monossaturadas e polinsaturadas totais, fibra e Kcal totais, considerando os parâmetros da Sociedade Brasileira de Cardiologia[44].

As voluntárias foram submetidos, a coleta de sangue, após jejum de 12 horas. Foram coletados 5mL de sangue em tubos com EDTA, e após a coleta foram centrifugados a uma velocidade de 3.000 rotações/min por 10 minutos.

O sangue para análise foi coletado em dois momentos, dia basal e dia experimento, em ambos os grupos, após jejum de 12 horas, o sangue foi centrifugado, o soro foi aliquotado e congelado a –80 ºC para posterior análise. As análises de soro foram feitas da seguinte maneira:

As carbonilas - As amostras de sangue foram incubadas com 2,4 dinitrofenilhidrazina (DNPH 10 mmol / L) em solução de 2,5 mol / L HCl durante 1 h à temperatura ambiente, no escuro. As amostras foram submetidas a vortex a cada 15 min. Em seguida, adicionou-se 20%

de solução de TCA (p / v) em amostras de tubos, deixou-se em gelo durante 10 min e centrifugou-se durante 5 min a 1000g, para recolher precipitados de proteína. Outra lavagem foi realizada com 10% de TCA. O sedimento foi lavado 3 vezes com etanol: acetato de etilo (1: 1) (v / v). Os precipitados finais foram dissolvidos em solução de cloridrato de guanidina a 6 mol / L, deixados durante 10 minutos a 37 ° C e lidos a 360 nm. (Reznick e Parker, 1994)[45] Os resultados foram expressos como nmol / mg prot.

Para análise das sulfidrilas as amostras foram incubadas por 15 min, centrifugadas a 1800g por 15 min e lidas no comprimento de onda de 412nm. Foram utilizadas 100uL da amostra adicionados 300uL de tris 0,002mol/L, pH=8,2 e 20uL de solução de DTNB 0,01mol/L. O cálculo das sulfidrilas foi baseada na metodologia de Sedlak e Lindsau[46] e seguiu a fórmula: Sulfidrilas = (abs x diluição) / (13100x proteína).

Para avaliação da peroxidação lipídica foram mensuradas substâncias reativas ao ácido tiobárbiturico (TBARS). O método utilizado se baseia na reação de duas moléculas de ácido tiobarbitúrico com uma de malondialdeído (MDA), produzindo um complexo de coloração rósea que pode ser quantificado pela leitura em espectrofotômetro em um comprimento de onda de 532nm.

Foi adicionado a uma alíquota de 50 µL de plasma, 12,5 µL de SDS (8,1%), 93,75uL de ácido acético (20%), pH 3,5, 93,75 uL de ácido tiobarbitúrico (0,8%). Foi agitada e encubada em banho fervente por 1h. Foi resfriado em temperatura ambiente, centrifugado a 3500rpm. O sobrenadante foi lido em espectofotômetro a tempertatura ambiente em um comprimento de onda de 532nm.

A etapa inicial para análise dos ácidos graxos foi a transesterificação das amostras através etapas sucessivas.

A primeira etapa foi a de extração seguida da etapa de hidrólise e esterificação. Da mesma forma os padrões à 99% de pureza dos ácidos graxos (pelargônico, azeláico, oleico e elaídico) também foram transesterificados nas distintas etapas.

Após a transesterificação dos padrões e amostras os mesmos foram analisados pela cromatografia gasosa[47] com aparelho Thermo Scientific, modelo GC, Focus Séries, N° de série:10902047 e o metanol foi utilizado como solvente.

A temperatura máxima utilizada para condicionamento da coluna foi de 230ºC com tempo de leitura de 21.78min e rampa de 30ºC/min [48].

A identificação dos padrões procedeu-se com a leitura isolada dos mesmos em 21.78minutos, para determinação dos seus respectivos tempos de retenção e área, seguido pela leitura simultânea, confeccionando um único padrão.

O estudo foi submetido ao Comitê de Ética em Pesquisa da Faculdade Adventista da Bahia e foi aprovado sob o protocolo 34017514.5.0000.0042. Durante todo o estudo foram observadas as diretrizes sobre a pesquisa com seres humanos da Resolução 466/2012 do Conselho Nacional de Saúde.

Os dados foram analisados previamente quanto a simetria pelo teste de Shapiro-Wilk, *Kolmogorov-Smirnov*, simetria, kurtose e histograma. Para a caracterização das variáveis idade, IMC, HOMA-IR, HOMA–beta, Insulina, glicemia, TG, CT, HDL, LDL, TG/HDL, vitaminas, minerais, TBARS, Sulfidrilas, Carbonilas, Proteína C Reativa de alta sensibilidade e ácidos graxos foi utilizada a média e o desvio padrão ou mediana e o intervalo interquartil a depender do comportamento da variável.

Para a comparação dos efeitos do exercício sobre percentual de ácidos graxos (Pelargônico, azeláico, elaídico e Oleico), perfil glicêmico, perfil lipídico, e estresse oxidativo do soro de mulheres com aumento de massa corporal foram feitas avaliações inter e intragrupo, com a utilização do teste t de Student pareado e não pareado em casos de simetria e Mann-Whitney e Wilcoxon Sign-Rank em caso de dados não paramétricos.

Foram feitas correlações de Spearman entre a PCR e fatores preditores metabólicos e alimentares. As variáveis que apresentaram significância estatística (glicose, insulina, índice de Homa, IMC, consumo de gorduras poliinsaturadas totais e de fibras) foram incluídas no modelo de regressão logística. Foi testada a calibração do modelo pelo teste de Homer e Lemershow e o mesmo apresentou-se calibrado ($p=0,07$). O nível de significância foi definido por valor de $p<0,05$. Os dados foram analisados com o uso do software Statistical Package for the Social Science (SPSS) versão 14.0.

5 ÍNDICE DOS ARTIGOS CIENTÍFICOS

5.1 Artigo 2 - Influence of subacute physical exercise on medium and long chain fatty acid profile in serum of individuals with increased body weight.

*Nutrición Hospitalaria**, em revisão.***

Title: Influence of subacute physical exercise on medium and long chain fatty acid profile in serum of individuals with overweigth

Authors: Djeyne Silveira Wagmacker, M.Sc[1,2],Jefferson Petto, M.D, PhD[1,2] Amanda Silva Fraga[2], Jackeline Barbosa Matias[1], Sindy Kerole Andrade Mota[1], Luiz Erlon Araujo Rodrigues, M.D, PhD[2] and Ana Marice Ladeia, M.D, PhD[2].

College of Adventist Bahia, Bahia Brazil[1] Bahian School of Medicine and Public Health, Science Development Foundation of Bahia, Bahia, Brazil.[2]

Corresponding author: Djeyne Silveira Wagmacker
Current address: Br 101, Km 197, cx postal 18, Salvador, Bahia, Brazil
e-mail: djeyne@hotmail.com.br

Original article

Keywords: Obesity, Exercise, Fatty Acids

Running title: Influence of exercise subacute and fatty acid

Word count: 3879 words

Clinical trial registration: NCT03170973

Funding: This study was financed by the National Council for Scientific and Technological Development (CNPQ)

Acknowledgments: This work was supported by National Council for Scientific and Technological Development (CNPQ)

Disclosure: The authors declare there is no pertinent conflict of interest.

WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT?

- Chronic effect of the exercise on the lipid profile

WHAT DOES THIS STUDY ADD?

- No clinical trial evaluating the acute effect of physical exercise based on energy expenditure on the acids fatt of women with altered body weight was identified.
- The results on the effect of acute exercise on metabolism have not yet been fully elucidated with divergent conclusions.

INFLUENCE OF SUBACUTE PHYSICAL EXERCISE ON MEDIUM AND LONG CHAIN FATTY ACID PROFILE IN SERUM OF INDIVIDUALS WITH OVERWEIGTH

Wagmacker DS[1,2], Petto J[1,2], Moreira JB[1], Mota SKA[1], Rodrigues LEA[2], Ladeia AMT[1]

[1] FADBA - "Faculdade Adventista da Bahia", (Adventist Faculty of Bahia) [2] EBMSP-"Escola Bahiana de Medicina e Saúde Pública" (Bahiana School of Medicine and Public Health)

ABSTRACT

Objective: to test the hypothesis that acute physical exercise would change the fatty acids in the serum of individuals with increased body weight. **Method:** Included in the sample were 66 women with excess weight, (BMI = 29.6±4.2) sedentary, and aged 24.4±3.6 years, randomly divided into groups control and exercise. After 12 hours fasting, basal blood collection was performed. 12 hours after the first collection, the exercise group was submitted to a physical exercise session with energy expenditure of 250Kcal. The volunteers underwent a second blood collection 24 hours after the first and dosed the fatty acids: pelargonic, azelaic, elaidic and oleic. **Results**: Physical exercise did not changes the fatty acid profile response for both the intragroup analysis and intergroup analysis: Exercise Group: Pelargonic (before = 0.12±0.06 % Vs. after = 0.15±0.14%, p=0.507); Azelaic (before = 20.3±10.5 % Vs. after = 27.7±25.4%, p=0.295); Elaidic (before=0.03±0.01% Vs. after=0.04±0.01%, p=0.328); Oleic (before=16.1±7.4% Vs. after=20.3±14.6%, p=0.236); Control Group: Pelargonic (before=0.70±0.45% Vs. after=0.71±0.51%, p=0.776); Azelaic (before=62.1±26% Vs. after=57.1±27%, p=0.197); Elaidic (before= 0.05±0.02% mg/dL Vs. after=0.05±0.03%, p=0.530); Oleic (before=26.8±22.7% mg/dL Vs. after=29.0±22.4%, p=0.525). **Conclusion**: women with overweight, low intensity physical exercise is not capable of changes the medium chain fatty acids in the first 12 hours.

Key Words: Obesity, Exercise, Fatty Acids.

INTRODUCTION

Fatty acids, the constituent elements of lipids, are organic components that contain carbon and hydrogen in their molecules. When short and medium chain fatty acids (C4-C12) are not supplied by the diet, they are synthesized, mainly in the cytoplasm of hepatic and adipose tissue cells. The long chain fatty acids (C12-C24) are supplied by the diet. (1)

The process of fatty acid uptake and oxidation is of particular metabolic significance, both at rest and during light to moderate exercises. The fatty acids are the predominant source of substrate for ATP resynthesis. During aerobic exercise, there is an increase in the release of fatty acids from the adipose tissue to accommodate the increased energy needs of the exercise(2). As the exercise progresses, long chain fatty acids, provided by the blood or from hydrolysis of intramuscular triacylglycerols, are metabolized to generate energy. The supply of fatty acids from the hydrolysis of intramuscular triacylglycerols is limited and during exercise, the myocytes consume approximately 90% of the free fatty acids derived from blood plasma (3).

Studies have also suggested that physical exercise performed in an acute manner promotes changes in the fatty acid transport genes, preceding increases in RNAm expression (4). This allows optimal delivery of fatty acids to the transporters, and their greater metabolism, although this latter occurrence has not yet been tested (5).

The use of lipids is modulated by the availability of fatty acids in plasma andthe obesity may influence the increase in plasma AG levels (6). Glycolytic activity is changed in this population, and lipid metabolism becomes a preferential pathway. In obese subjects, the metabolic responses of fatty acid mobilization appear to be favored by aerobic activity; however, the responses are not yet conclusive (7).

Therefore, the aim of this study was to test the hypothesis that acute physical exercise would change the medium and long chain fatty acids in the serum of individuals with increased body weight.

METHODS:

Study Design and Population

This Randomized Clinical Trial, Registered in Clinical Trial under Protocol NCT03170973, was conducted with a population accessible to the School Clinic of the Adventist Faculty of Bahia, Brazil. Data collection was performed during the period from September 2015 to May 2016.

All the women registered with the Physical Therapy Service of the Clinic School who had a body mass index (BMI) above 24.99kg/m^2 were invited to participate in the study. A total of 66 volunteers fulfilled the inclusion criteria, which were: age between 18 to 30 years, BMI>24.99kg/m^2 and sedentarism. Sedentarism was determined based on the International Physical Activity Questionnaire - long version (8). Excluded from the study were women who presented with cardiovascular and metabolic disease, hypothyroidism, renal parenchymal disease or diabetes mellitus, history of alcoholism or smoking, use of hypolipemiant, corticosteroid, diuretic, beta-blocker, and contraceptive medications.

The women were randomly divided into two groups: exercise and control; both with 33 volunteers.

Exercise Group

After 12 hours fasting, the volunteers were submitted to blood collection, in the antecubital vein, to measure the basal serum values of triglycerides, total and fractionated cholesterol, glycemia and insulin. From the Glycemia and Insulin values, the Homa-IR and Homa-Beta index values were calculated by means of the equation proposed by Mathews (9).

When 12 hours had elapsed after the first blood collection, the patients performed a physical exercise session on an ergometric treadmill. This was divided into 3 time intervals: warming-up, conditioning and cooling down. The duration of warming-up was 7 minutes; cooling off 5 minutes, and conditioning time corresponded to energy expenditure of 250Kcal (10) with light intensity based on the Borg (11) rating of perceived exertion, that is, on the original scale - a value between 9 and 11. For better understanding of this scale, on the day before the exercise, the volunteers were familiarized with the BORG concept to allow them to get used to providing adequate answers when they were asked about the intensity of the exercise. A cardiac frequency meter was used, which measured the energy expenditure based on the volunteer’s body mass, sex and age.

After the physical exercise session, the volunteers were instructed to go home and keep to their habitual diet. When 24 hours had elapsed after the first blood collection, the volunteers returned to the laboratory after a 12-hour fast, and once again had blood samples collected. The diet of the previous two days before the blood exam was evaluated by means of a 24 hours diet diary.

Control Group

The women in the control group were submitted to the same data collection protocol as that of the experimental group, however, they did not do the exercise 12 hours after the first blood collection. They were instructed not to perform any physical exercise on the two days before the blood collection, as shown in the flow diagram presented in Figure 1.

24 Hour Diet Diary

Once again, they were evaluated as regards their diet on the day before the exam by means of keeping a 24-hour food-intake diary. The 24-hour food intake diary was evaluated by means of an interview held at the time of blood collection, in which the volunteers informed what they had consumed on the day before the interview, in the three main meals, and between these meals. The instrument was applied by the same examiner, and to facilitate responses, home measurements were used (12). Quantitative evaluation of the diet was performed by using the software *Avanutri Revolution.* For the purposes of analysis the consumption of the following were considered: micronutrients (vitamins and minerals), cholesterol, total saturated, monosaturated and polyunsaturated fats and total dietary fiber, using the parameters of the Brazilian Society of Cardiology (13).

Blood Collection and Fatty Acid Analysis

The volunteers were submitted to blood collection after fasting for 12 hours. Samples of 5 ml of blood were collected in tubes with EDTA, and after collection the samples were centrifuged at a speed of 3,000 rpm for 10 minutes.

Fatty acids, which have 9C (Azelaic and Pelargonic) and 18C (oleic and elaidic) carbon molecules tested in this study are considered medium and long chain fatty acids and would be able to be more easily metabolized in the mitochondria for energy production. In addition to this aspect, oleic acid is a fatty acid highly

stimulated for consumption as it becomes interesting to understand how physical exercise interferes in this fatty acid as well as its geometric isomer (Elaídico) and its by-products of metabolism (Azelaic and Pelargonic). (14)

The initial stage for fatty acid analysis was trans-Esterification of the samples by means of successive stages. The first stage was extraction then the hydrolysis and esterification stages began. In the same way, the patterns at 99% Purity of the fatty acids (Pelargonic, azelaic, Oleic and elaidic) were also trans-esterified in the different stages. (15)

After trans-esterification of the patterns and samples these were analyzed by gas chromatography (16) by means of the Thermo Scientific appliance, model GC, Focus Series, Serial N°:10902047, and methanol was used as solvent.

The maximum temperature used for conditioning the column was 230ºC with readout time of 21.78 minutes and ramp of 30ºC/min (17).

Identification of the patterns proceeded with isolated readout of the samples in 21.78 minutes, to determine their respective retention times and area, followed by simultaneous readout, to construct a single pattern.

The retention times (t) and area (a) of the pelargonic, azelaic, elaidic and oleic acids were: 5.9 minutes and 537,138,819; 7.8 minutes and 113,433,890; 9.4 minutes and 1,274,291,989; 12.9 minutes and 75,971,297 respectively.

Ethical Aspects

This study was submitted to the Research Ethics Committee of the Faculdade Adventista da Bahia and approved under Protocol N° 34017514.5.0000.0042. Throughout the entire study, the guidelines on research with human beings of Resolution 466/2012 of the National Health Council were observed.

Statistical Analysis

The data were previously analyzed by the Shapiro-Wilk test, with regard to symmetry. For characterization of the following variables: BMI, age, HOMA-IR, HOMA–beta, Insulin, glycemia, TG, CT, HDL, LDL and TG/HDL, vitamins, minerals and fatty acids, the mean and standard deviation or median and interquartile interval were used, depending on the behavior of the variable. The level of significance was defined by the value of $p<0.05$.

For comparison of the effects of exercise on the percentage of fatty acids (pelargonic, azelaic, elaidic and oleic) in the serum of obese women, inter- and intragroup comparisons were made by using the paired and non-paired Student's-*t* test in cases of symmetry. The data were analyzed by using the Statistical Package for the Social Sciences (SPSS) software program, version 24.0

RESULTS

In Table 1 the authors observed that no differences were found when the clinical, anthropometric and lipid profile variables were compared. The insulin, insulin resistance and insulin sensitivity values were observed to be more elevated in the control group.

In Table 2 the intra-group analysis of the percentages of fatty acids contained in the serum of women with increased body weight may be observed. No differences were found when the percentages of fatty acids before and afterwards were compared, in the Control and Exercise Groups.

When the intergroup analysis was performed of the differences (after - before) in the percentages of fatty acids contained in the serum of women with increased body weight, the authors observed that no differences were found between the Control and Exercise Groups. (Table 3)

DISCUSSION

In our studies, there was no evidence of differences in the percentages of plasma medium chain fatty acids after low intensity physical activity. Studies have suggested that acute physical exercise promoted changes in the fatty acid transport genes, preceding increases in RNAm expression (4) and that the change in these transporters favored greater fatty acid metabolization (5). Moreover, it is possible that exercise changes the composition of the fatty acids in plasma (18). In our study, we observed that the medium and long chain fatty acids present in the plasma of individuals with increased weight did not undergo changes 12 hours after the practice of low intensity physical exercise.

The process of fatty acid uptake and oxidation is of significant importance, both at rest and during light to moderate exercises. The fatty acids are the predominant source of substrate for ATP resynthesis (2). Oxidative metabolism

allows energy to be obtained from fatty acids in an intramitochondrial localization. Thus, to enable acyl-CoA to be used by it (oxidative metabolism), it is necessary to overcome the impermeability of the external and cytoplasmic membrane of the mitochondria to attain the acyl-CoA. The enzyme responsible for this transport is Carnitine-CoA acyltransferase (Carnitine O-Palmitoyltransferase). This enzyme presents greater specificity for Palmitoyl-CoA, however, it catalyzes transport of fatty acids with a carbonated chain length between C4 and C18. Fatty acid chains longer than these are more difficult to be transported. Once within the mitochondria, acyl-CoA may be used in the lipolytic metabolism of LYNEN.(12) The fatty acids, which have 9C (Azelaic and Pelargonic) and 18C carbon molecules (oleic and elaidic), tested in this study are considered medium and long chain fatty acids, and would have conditions to be more easily metabolized in the mitochondria for energy production. These fatty acids would be expected to present reduced percentages after physical activity.

Different studies have been presented relating the effect of physical exercise on lipids, however the results are still controversial (7;19;20;21). Over the last few decades growing evidence could be observed that acute physical exercise could have a beneficial effect on the lipid profile (22;23;24), and it has also been observed that its effect could last for up to 48 hours after the exercise session (25). It is worth pointing out that the majority of studies have evaluated the late effect of acute exercise on lipids after time intervals of 12, 24 and 48h. The difficulty with analyses and interpretation of these studies lies in the use of different physical activity protocols established(26).

In studies that have evaluated the drop in triglycerides in the post-prandial period, the calorie expenditure with physical exercise was the determinant factor in the magnitude of the drop in triglycerides. These studies suggested that very low intensities might not present significant results, due to the fact that they do not attain an adequate calorie expenditure to cause a reduction in the triglycerides (27;28). Contradicting studies that suggested the practice of low intensity activity for better effect on oxidation of the fats(7; 29).

As observed in Table 1, the Control Group presented a higher insulin index when compared with the Exercise Group. Since the increase in insulin could limit the mobilization of fatty acids (30), greater fatty acid mobilization was to be expected in the Exercise Group. However, in spite of the lower insulin values in the Exercise

Group, this aspect did not favor greater fatty acid mobilization, because no differences were observed in the Pelargonic, azelaic, Oleic and elaidic values between the Control and Exercise Groups.

Among the limitations observed in this study, the following are included: a single time interval of observation of the responses related to fatty acids 12 hours after exercise; and absence of caloric expenditure above 250Kcal for different comparisons. With reference to physical activity, factors such as calorie expenditure and time of observation may have a strong influence on the metabolic responses (31). This study presented the responses to a calorie expenditure and specific time, and for these variables, no change were observed in the different fatty acid values, which cannot be affirmed for time intervals longer than 12 hours, and calorie expenditures different from 250Kcal. Different physical exercise protocols must be investigated to contribute further elucidation about the effects of physical activity on the medium and long chain fatty acids.

CONCLUSION

According to the results of this study, low intensity physical exercise does not change the medium and long chain fatty acids, in a subacute manner, in overweight women.

ACADEMIC CONNECTION

This research is part of the doctoral thesis of Djeyne Silveira Wagmacker, at the Bahiana School of Medicine and Public Health, Salvador, BA – Brazil.

REFERENCES

(1) Kadhum AA, Shamma MN. Edible lipids modification processes: A review Crit Rev Food Sci Nutr. 2017 Jan 2; 57(1):48-58. DOI: 10.1080/10408398.2013.848834

(2) Jayewardene AF, Mavros Y, Reeves A, Hancock DP, Gwinn T, Rooney KB. Interactions Between Fatty Acid Transport Proteins, Genes That Encode for Them, and Exercise: A Systematic Review. J Cell Physiol. 2016 Aug; 231(8):1671-87. DOI: 10.1002/jcp.25281.

(3) Harasim E, Kalinowska A, Stępek T, Chabowski A. The role of fatty-acid transport proteins (FAT/CD36, FABPpm, FATP) in lipid metabolism in skeletal muscles. Postepy Hig Med Dosw. (Online). 2008; 62: 433-441.

(4) Barres R; Yan J; Egan B; Treebak JT; Rasmussen M; Fritz T. et al. Acute exercise remodels promoter methylation in human skeletal muscle. Cell Metab 15:405–411. 2012 Mar 7; 15(3):405-11. DOI: 10.1016/j.cmet.2012.01.001

(5) Egan B, Zierath JR. Exercise metabolism and the molecular regulation of skeletal muscle adaptation. Cell Metab. 2013 Feb 5;17(2):162-84. DOI: 10.1016/ j.cmet. 2012 .12.012.

(6) Jongh RT, Serné EH, Ijzerman RG, Vries G, Stehouwer CDA. Free fatty acid levels modulate microvascular function. Diabetes. 2004;53:2873-82.

(7) Jabbour G, Iancu H-D, Paulin A, Lavoie J- M, Lemoine-Morel S, Zouhal H. Effects of Acute Supramaximal Cycle Exercise on Plasma FFA Concentration in Obese Adolescent Boys. PLoS One. 2015 Jun 15; 10(6): e0129654. DOI:10.1371/journal.pone.0129654 .

(8) Matsudo S; Araújo T; Marsudo V; Andrade D; Andrade E; Oliveira LC; et al. Questinário internacional de atividade física (IPAQ): estudo de validade e reprodutibilidade no Brasil. Rev. bras. ativ. fís. saúde; 2001; 6(2):05-18.

(9) Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia, 1985; 28:412-9.

(10) Plaisance, EP. Mestek, ML. Mahurin, AJ. Postprandial triglyceride responses to aerobic exercise and extended-release niacin[1-3]. Am J Clin Nutr. 2008; 88(4):30-37.

(11) Chen MJ, Fan X, Moe ST. Criterion-related validity of the Borg ratings of perceived exertion scale in healthy individuals: a meta-analysis. J Sports Sci 2002; 20:873-99.

(12) Fisberg RM, Villar BS. Manual de receitas e medidas caseiras para cálculo de inquéritos alimentares. São Paulo: Signus, 2002.

(13) Santos R.D., Gagliardi A.C.M., Xavier H.T., et al. Sociedade Brasileira de Cardiologia. I Diretriz sobre o consumo de Gorduras e Saúde Cardiovascular. Arq Bras Cardiol. 2013; 100(1Supl.3): 1-40.

(14) Mäkinen S, Nguyen HY, Skrobuk P, Koistinen HA. Palmitate and oleate exert differential effects on insulin signalling and glucose uptake in human skeletal muscle cells. Endocr Connect. 2017 Jun 5. pii: EC-17-0039. DOI: 10.1530/EC-17-0039.

(15) Bligh, EG; Dyer WJ. A rapid method of total lipid extraction and purification. Canadian Journal Biochemistry Physiological. 1958; 27(8): 911-917.

(16) Garelnabi M, Litvinov D, Parthasarathy S. Evaluation of a gas chromatography method for azelaic acid determination in selected biological samples. North

American Journal of Medical Sciences. 2010; 2(9): 397-402. DOI:10.4297/najms.2010.2397.

(17) Miyamoto S, Taylor SL, Barupal DK, Taguchi A; Wohlgemuth G; Wikoff WR; et al. Systemic Metabolomic Changes in Blood Samples of Lung Cancer Patients Identified by Gas Chromatography Time-of-Flight Mass Spectrometry. Yeung PKF, ed. Metabolites. 2015; 5(2): 192-210. DOI:10.3390/metabo5020192.

(18) Lee S; Norheim F; Gulseth HL; Langleite TM; Kolnes KJ; Tangen DS. Interaction between plasma fetuin-A and free fatty acids predicts changes in insulin sensitivity in response to long-term exercise. *Physiol Rep. 2017; 5(5):* e13183. DOI:10.14814/phy2.13183.

(19) Bilet L, Brouwers B, Van Ewijk PA, Hesselink MKC; Kooi ME; Schrauwen P. Acute exercise does not decrease liver fat in men with overweight or NAFLD**.** Sci Rep. 2015 ; 13(5):9709. DOI: 10.1038/srep09709.

(20) Thivel D, Metz L, Julien A, Morio B, Duche P. Obese but not lean adolescents spontaneously decrease energy intake after intensive exercise. Physiol Behav 2014; 123: 41–46. DOI: 10.1016/j.physbeh.2013.09.018.

(21) Bodel NG, Gillum T. 90 Minutes of Moderate-Intensity Exercise does not Attenuate Postprandial Triglycerides in Older Adults. Int J Exerc Sci. 2016; 9(5): 677-684.

(22) Durstine JL, Ferguson MA, Szymanski LM, et al. Effect of single session exercise on lipoprotein (a). Med Sci Sports Exerc.1996;28:1277-81.

(23) Harrison M; O'Gorman DJ; McCaffrey N; Hamilton MT; Zderic TW; Carson BP; et al. Influence of acut exercise with and whithout carbohydrate replacement on postprandial lipid metabolism. J Appl Phisiol. 2009;106(3):943-9. DOI: 10.1152/japplphysiol.91367.2008.

(24) Davitt PM, Arent SM, Tuazon MA, Golem DL, Henderson GC. Postprandial triglyceride and free fatty acid metabolism in obese women after either endurance or resistance exercise. J Appl Physiol. 2013; 114:1743–54. DOI: 10.1152/japplphysiol.00095.2013.

(25) Durstine JL, Thompson PD. Exercise in the treatment of lipid disorders. Cardiol Clin 2001;19:471-88.

(26) Thivel D; Rumbold PL; King NA; Pereira B; Blundell JE; Mathieu M-E. Acute post-exercise energy and macronutrient intake in lean and obese youth: a systematic review and meta-analysis. International Journal of Obesity. 2016; 40(10):1469–1479. DOI:10.1038/ijo.2016.122

(27) Ferreira AP, Ferreira CB, Souza VC, Córdova COA; Silva GCB; Nóbrega OT; et al. The influence of intense intermittent versus moderate continuous exercise on postprandial lipemia. Clinics. 2011; 66(4): 535-41. DOI: 10.1590/S180759322011000400003

401 (28) Katsanos CS, Grandjean PW, Moffatt RJ. Effects of low moderate exercise
402 intensity on postprandial lipemia and postheparin plasma lipoprotein lipase activity in
403 physically active men. J Appl Physiol 2004;96:181-8.

404 (29) Vistisen B, Nybo L, Xu X, Hoy C-E, Kiens B. Minor amounts of plasma medium-
405 chain fatty acids and no improved time trial performance after consuming lipids. J
406 Appl Physiol. 2003; 95(6):2434-43.

407 (30) Hall GV. The Physiological Regulation of Skeletal Muscle Fatty Acid Supply and
408 Oxidation During Moderate-Intensity Exercise. Sports Med. 2015; 45 (Suppl 1):S23–
409 S32. DOI: 10.1007/s40279-015-0394-8.

410 (31) Petto J, Batista ARMS, Oliveira CS, Ladeia AMT. Efeito agudo do exercício
411 físico na lipemia pós-prandial: Revisão. Rev. Bras. Fisiol. Exercício. 2012.11:111-4.

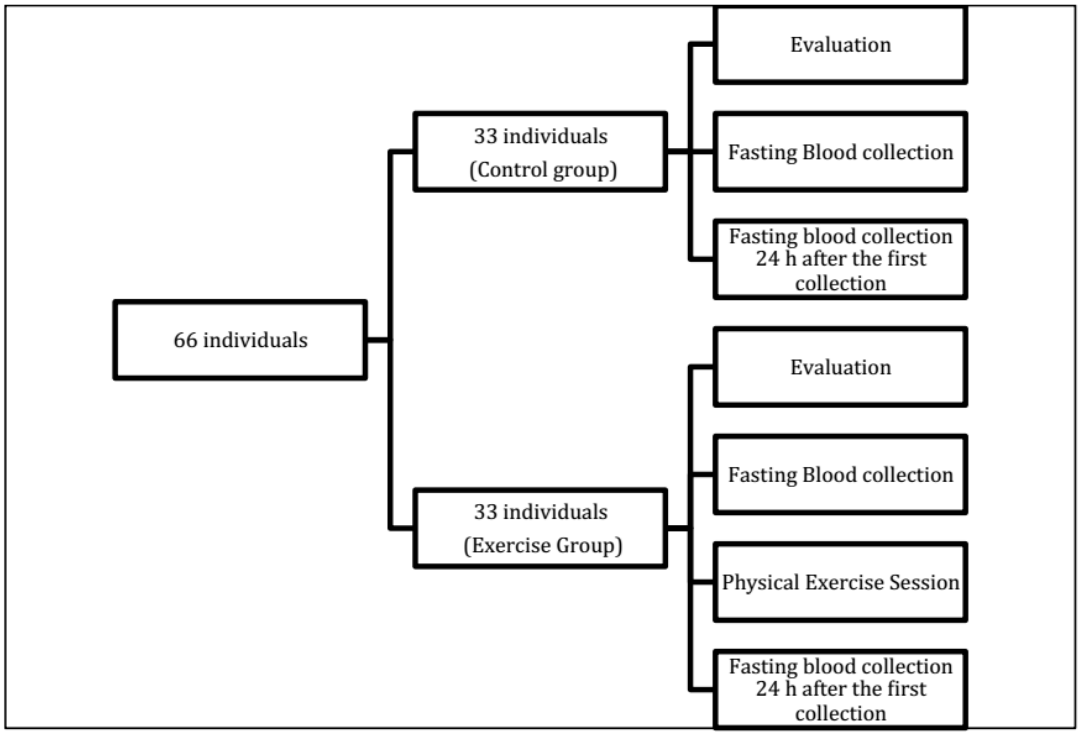


Figure 1 - Flow Diagram showing Data Collection

Table 1 Clinical and anthropometric characteristics of the sample per control and exercise group, on the first day of blood collection, n=66.

VARIABLES	CG (n=33)	EG: (n=33)	p
Age	23 ± 4	25 ± 3	0.25
Body Mass Index	30 ± 4	29 ± 4	0.44
Triglycerides (mg/dL)	99 ± 43	102 ± 64	0.81
Total Cholesterol (mg/dL)	163 ± 29	159 ± 30	0.59
High Density Lipoprotein (mg/dL)	45 ± 8	49 ± 10	0.11
Low Density Lipoprotein (mg/dL)	97 ± 24	89 ± 26	0.18
TG/HDL	2 ± 1.4	2.2 ± 1.6	0.897
Glycemia (mg/dL)	84 ± 9	82 ± 8	0.22
Insulin (mcIU/mL)	12 ± 6	8 ± 5	0.01*
Homa IR	2.9 ± 1.4	2.0 ± 1.3	0.01*
Homa-Beta	40 ± 19	27 ± 18	0.01*

CG–Control Group; EG–Experimental Group; Mean±Standard Deviation Student's-*t* Test;

Table 2 – Intra-group analysis of the percentages of fatty acids contained in the serum of women with increased weight, before and after, in the Exercise and Control Groups.

	BEFORE	AFTER	p
GE(n=33)			
Pelargonic (%)	0.12 ±0,06	0.15±0,14	0.507
Azelaic (%)	20.3 ± 10,5	27.7±25,4	0.295
Elaidic (%)	0.03±0,01	0.04±0,02	0.328
Oleic (%)	16.1±7,4	20.3±14,6	0.236
GC(n=33)			
Pelargonic (%)	0.70±0,45	0.71±0,51	0.935
Azelaic (%)	62.1 ± 26	57.1± 27	0.197
Elaidic (%)	0.05±0,02	0.05±0,03	0.530
Oleic (%)	26.8±22,7	29.0±22,4	0.525

CG–Control Group; EG–Exercise Group; Mean±Standard Deviation; Paired Student's-t test.

Table 3 – Analysis of **Δ** (value % after- value % before) of medium and long chain fatty acids in the Control and Exercise Groups, n=33.

	GC	GE	Valor de p
Δ Pelargonic (%)	0,02 ±0,12	0,01±0,33	0,984
Δ Azelaic (%)	7,34±28,84	-5,01±16,32	0,548
Δ Elaidic (%)	0,01±0,02	0,01±0,04	0,952
Δ Oleic (%)	4,17±14,41	0,01±27,4	0,356

GC–Control Group; GE–Exercise Group; Mean±Standard Deviation; Student's-t test.

5.2 Artigo 3 - Metabolic reponses to a physical exercise session in women with excess body mass: randomized clinical trial. ***Lipids in Health and Disease****, 2017; 16:249.* DOI: 10.1186/s12944-017-0600-9.

Wagmacker *et al. Lipids in Health and Disease* (2017) 16:249
DOI 10.1186/s12944-017-0600-9

Lipids in Health and Disease

RESEARCH Open Access

Metabolic Reponses to a physical exercise session in women with excess body mass: randomized clinical trial

Djeyne Silveira Wagmacker[1,2,4*], Jefferson Petto[1,2], Amanda Silva Fraga[2], Jackeline Barbosa Matias[1], Sindy Kerole Andrade Mota[1], Luiz Erlon Araujo Rodrigues[2] and Ana Marice Ladeia[2,3]

Abstract

Background: There are various factors that influence the effect of physical exercise on the lipid profile, among them the body mass index and calorie expenditure of the exercise are some of the main factors. To test the hypothesis that a physical exercise session based on caloric expenditure may acutely modify the glycemia and lipid values of women with excess body mass.

Methods: The study included 66 women, randomly divided into two groups, control and experimental, with BMI of 29 ± 4.4 kg/m^2 vs 29 ± 4.3 kg/m^2 (p = 0.45) sedentary and aged 23 ± 3.8 vs 24 ± 3.5 years, respectively (p = 0.25). After 12 h fasting, the volunteers underwent the first blood collection. The experimental group was submitted to a physical exercise session corresponding to energy expenditure of 250Kcal, of light intensity based the Borg Rating of Perceived Exertion (RPE), 12 h after the first blood collection. The control and experimental group volunteers underwent a second blood collection 24 h after the first. Glycemia, insulin status and lipid profile were measured and Homa IR and Homa-beta were calculated. The t-test for independent and dependent samples was used, and a level of significance of 5% was adopted.

Results: Physical exercise changed the glycemic response in both the intragroup analysis (before = 96 ± 6.6 mg/dL vs after = 92 ± 6.6 mg/dL), (p = 0.01), and in the intergroup analysis (control = Δ 0.9 ± 6.1 vs experimental = Δ -4.1 ± 6.3) (p = 0.02). No changes were shown for the Homa IR, Homa Beta and Insulin indexes. When the lipid profiles were evaluated, differences in HDL were shown in the intragroup analysis (before = 89 ± 10.5 mg/dL vs. after = 91 ± 10.3 mg/dL) (p = 0.04). For the other parameters (LDL, TG, Total Cholesterol, TG/HDL), no changes were shown.

Conclusion: In women with excess body weight, a low intensity exercise session diminished the glycemia, but did not change the lipid response.

Trial registration: NCT03170973. Retrospectively registered.

Keywords: Obesity, Motor activity, Lipids, Glycemia

* Correspondence: djeyne@hotmail.com.br
[1]College Adventist Bahia, Cachoeira, Bahia, Brazil
[2]Bahian School of Medicine and Public Health, Science Development Foundation of Bahia, Bahia, Brazil
Full list of author information is available at the end of the article

What does this study add?

- No clinical trial evaluating the acute effect of physical exercise based on energy expenditure on the metabolism of women with altered body weight was identified.
- The results on the effect of acute exercise on metabolism have not yet been fully elucidated with divergent conclusions.

Background

Various studies have evaluated the acute and chronic effects of physical exercise on metabolic variables [1, 2]. The large majority of these studies have observed that physical exercise was an efficient therapy regulating both the lipid and glycemic profiles.

More specifically, the effects of a single session of physical exercise have also been the object of study of some researches [3–5]. Along this line, there is a strong counter point between the studies, since some have shown positive effects, while other pointed out no changes in the lipid or glycemic profiles with only one session [4, 5].However, all the studies that have evaluated the effect of a single exercise session on the metabolic response, raised the hypothesis that there are various factors that influence the results, such as: the clinical condition and previous functional capacity of the participants, and the characteristics of the exercise applied. Moreover, it is known that one of the clinical conditions with great influence on this response is the Body Mass Index (BMI). Furthermore, it has been demonstrated that obese persons with dyslipidemia or diabetes mellitus present more positive results when compared with eutrophic populations [6, 7]. Caloric expenditure has been pointed out as being the main variable of physical exercise that determines the beneficial effect on the lipid profile [8]. Thus, the aim of this study was to test the hypothesis that a physical exercise session based on caloric expenditure may acutely modify the variables of glycemia and lipid values of women with excess body mass index.

Methods

Study design and population

This was a Randomized Clinical Trial registered in the Clinical Trial Registry with the identifier number NCT03170973. The accessible population came from the Clinic School of the "Faculdade Adventista da Bahia", in Cachoeira, BA, Brazil.

All the women registered with the Physical Therapy Service of the Clinic School who had a body mass index (BMI) above 24.9 kg/m^2 were invited to participate in the study. A total of 66 volunteers fulfilled the inclusion criteria, which were: age between 18 to 30 years, BMI > 24.9 kg/m^2 and be sedentary. Sedentarism was determined based on the International Physical Activity Questionnaire - long version [9].

Excluded from the study were women who presented cardiovascular; metabolic disease; hypothyroidism; renal parenchymal disease or diabetes mellitus; history of alcoholism or smoking; use of hypolipemiant, corticosteroid, diuretic, beta-blocker, and contraceptive medications.

The women were randomly divided into two groups: exercise and control; both with 33 volunteers.

Exercise group

After 12 h fasting, the volunteers were submitted to blood collection, in the antecubital vein, to measure the basal serum values of triglycerides, total and fractionated cholesterol, glycemia and insulin. From the Glycemia and Insulin values, the Homa-IR and Homa-Beta index values were calculated by means of the equation proposed by Mathews and cols [10]. The patients were evaluated relative to diet on the two days before the blood exam, by means of a 24-h food intake diary, with a view to minimizing the effects of diet on the results.

When 12 h had elapsed after the first blood collection, the patients performed a physical exercise session on an ergometric treadmill. The exercise session was divided into 3 time intervals: warming-up, conditioning and cooling-off. The duration of warming-up was 7 min; cooling off 5 min, and conditioning time corresponded to energy expenditure of 250Kcal [11]with light intensity based on the Borg [12]. Rating of Perceived Exertion (RPE), that is, on the original scale - a value between 9 and 11. For better understanding of this scale, on the day before the exercise, the volunteers were familiarized with the RPE concept to allow them to get used to answering in an adequate manner when they were asked about the intensity of the exercise. A cardiac frequency meter was used, which measured the energy expenditure based on the volunteer's body mass, sex and age.

After the physical exercise session, they were instructed to go home and keep to their habitual diet. When 24 h had elapsed after the first blood collection, the volunteers returned to the laboratory after a 12-h fast, and once again had blood samples collected. Once again, they were questioned about their diet on the day before the exam by means of keeping a 24-h food-intake diary.

Control group

The women in the control group were submitted to the same data collection protocol as that of the experimental group, however, they did not do the exercise 12 h after the first blood collection. They were instructed not to perform any physical exercise on the two days before the blood collection, as shown in the flow diagram presented below. (Fig. 1).

Blood collection and metabolic profile

The volunteers were submitted to blood collection after fasting for 12 h. Samples of 5 ml of blood were collected in tubes with EDTA, and after collection the samples were centrifuged at a speed of 3000 rpm for 10 min.

Serum analyses were performed as follows: the blood glucose levels, triglycerides and total cholesterol were determined by the enzymatic calorimetric method; LDL was calculated by using the Friedwald [13] equation; and the Homa-IR and Homa-Beta indexes were calculated by using the equation proposed by Mathews and cols [10].

Ethical aspects

This study was submitted to the Research Ethics Committee of the Faculdade Adventista da Bahia and approved under Protocol No. 34017514.5.0000.0042. Throughout the entire study, the guidelines on research with human beings of Resolution 466/2012 of the National Health Council were observed.

Statistical analysis

The data were previously analyzed by the Shapiro-Wilk test, with regard to symmetry. For characterization of the following variables: BMI, age, HOMA-IR, HOMA–beta, Insulin, glycemia, TG, CT, HDL, LDL and TG/HDL, the mean and standard deviation or median and interquartile interval were used, depending on the behavior of the variable. The level of significance was defined by the value of $p < 0.05$.

For comparison of the effects of exercise on the glycemic, lipid and inflammatory profiles, inter- and intragroup comparisons were made by using the paired and non-paired Student's-t test in cases of symmetry, and the Mann–Whitney and Wilcoxon Signed-Rank tests in cases of non-parametric data. The data were analyzed by using the Statistical Package for the Social Sciences (SPSS) software program, version 14.0.

Results

This study included 66 young women, aged 24 ± 3.6 years, with BMI 29 ± 4.3Kg/m^2, with lipid and glycemic profiles within the values of normality.. Metabolic values do not differ between the experimental and control groups except for the insulin and Homa values that are higher in the control group. The clinical characteristics are described in Table 1.

In the intragroup analysis, a decrease in serum glycemia (96.7 ± 6.6 vs 92.6 ± 6.6 mg/dl) ($p = 0.01$) was observed in experimental group, only. No difference was demonstrated for the others variables in both groups (Table 2).

When the intergroup glycemic profile variation was analyzed, the decrease of glucose was lower in the experimental group. No difference was observed in insulin level, insulin resistance and insulin sensitivity between groups. (Table 3).

The intra-group analysis of lipid profile, showed a significant increase in the HDL values and a tendency to decrease in the TG/HDL ratio in the exercise group,

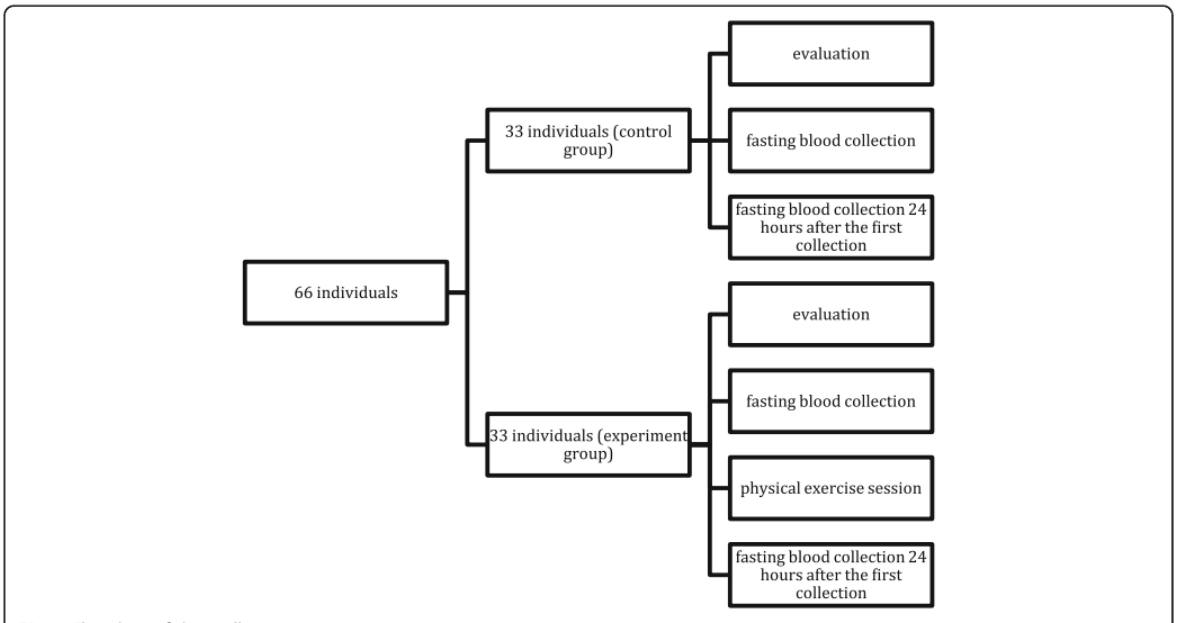


Fig. 1 Flowchart of data collection

Table 1 Clinical and anthropometric characteristics of the total sample and per group on the first day of blood collection

VARIABLES	Total Sample (n = 66)	CG (n = 33)	EG: (n = 33)	p
Triglycerides (mg/dL)	94 ± 43	99 ± 43	102 ± 64	0.81
Total Cholesterol (mg/dL)	162 ± 32	163 ± 29	159 ± 30	0.59
High Density Lipoprotein (mg/dL)	49 ± 10	45 ± 8	49 ± 10	0.11
Low Density Lipoprotein (mg/dL)	94 ± 28	97 ± 24	89 ± 26	0.18
Glycemia (mg/dL)	84 ± 8	84 ± 9	82 ± 8	0.22
Insulin (mcIU/mL)	10 ± 5	12 ± 6	8 ± 5	0.01*
Homa IR	2.4 ± 1.2	2.9 ± 1.4	2.0 ± 1.3	0.01*
Homa-Beta	34 ± 20	40 ± 19	27 ± 18	0.01*

CG-Control Group; EG-Experimental Group; *p<0.05, Student's-t Test

while in the control group, no change was found in any of the lipid profile variables (Table 4).

In the intergroup comparison, no difference was observed in the variation of triglycerides, total cholesterol, LDL-C, HDL-c and TG/HDL levels, neither in the experimental group nor in the control group (Table 5).

Dsiscussion

The results of this study demonstrated that low intensity physical exercises in women with excess weight, acutely reduced the serum glycemia, however, it did not change the lipid profile.

Some studies, conducted with other populations and different protocols have corroborated our results and pointed out that/?this type of?/exercise was incapable of improving the lipid profile in an acute manner [4]. However, in the study conducted by Ferguson et al. [14], the correspondence was investigated, between the energetic threshold and the possible changes in the triglyceride levels and concentrations of lipoproteins in trained men after four exercise protocols. The protocols were carried out with caloric expenditures of 800, 1100, 1300 and 1500 kcal. Twenty-four hours after performing the sessions, the HDL was significantly elevated in the exercises with expenditure of 1100, 1300 and 1500 kcal. Whereas the LDL concentration diminished significantly with an expenditure of 1300 kcal; and that of triglycerides, with 800 kcal after one single exercise session. In the same study, it was possible to observe an increase in lipoprotein lipase activity 24 h after the sessions with caloric expenditure of over 1100 kcal, and this remained elevated up to 48 h after the session using 1500 kcal, as these changes coincided with the changes in HDL. In another study, Ferreira et al. [15] also observed significant reduction in post-prandial lipemia in men submitted to different intensities of effort both with caloric expenditure of 500 kcal. They verified that both moderate and high intensity exercise presented reduction in post-prandial lipemia. Possibly the caloric expenditure on performing the protocol of this study was not enough to promote these changes.

Nevertheless, the protocol used was effective in reducing glycemia. The knowledge that exercise increases insulin sensitivity,, in both the acute and chronic form, served as a basis for explaining the results obtained in this study [16].

Some are the effects promoted by exercise, which explain this result. Physical exercise is known to increase the phosphorylation of insulin receptors (IRS1 and 2), which consequently facilitates the action of insulin [17]. This effect occurs during exercise and may last of up to 16 h after the exercise [18].

Table 2 Intragroup analysis of glycemic values, insulin and HOMA IR and HOMAbeta

	Before	After	P
EG (n = 33)			
Glucose (mg/dL)	97 ± 6.6	93 ± 6.6	0.01*
Insulin (mcIU/mL)	8 ± 5.2	8 ± 5.2	0.99
Homa Index	2.0 ± 1.3	1.9 ± 0.9	0.69
Homa-Beta	27.9 ± 18.7	27.6 ± 18.7	
CG (n = 33)			
Glucose (mg/dL)	97 ± 8.6	98 ± 8.8	0.41
Insulin (mcIU/mL)	12 ± 5.6	12 ± 5.2	0.78
Homa IR	2.9 ± 1.4	2.8 ± 1.3	0.76
Homa-Beta	40.7919.7	39.4 ± 17.1	0.70

EG = Experimental Group; CG = Control Group; *Bidirectional Student's-t Test for paired samples

Table 3 Comparison of variation in the glycemic profile in the Control and Exercise Groups

	CG (n = 33)	EG (n = 33)	p
Δ Glucose	0.90 ± 6.1*	−4.18 ± 6.3	0.02*
Δ Homa-IR	−0.06 ± 1.2*	−0.06 ± 0.9	0.99
Δ Homa-Beta	−1.30 ± 19*	−0.30 ± 13.8	0.81
Δ Insulin	0.00 ± 5.3*	−0.18 ± 3.8	0.87

EG = Experimental Group; CG = Control Group; *Bidirectional Student's-t Test for independent samples

Table 4 Intragroup Lipid Profile Analysis (n = 33)

	Before	After	p
Experimental			
Total Cholesterol (mg/dL)	159 ± 30.3	161 ± 34.0	0.36
Triglycerides (mg/dl)	102 ± 64.4	93 ± 49.3	0.08
High Density Lipoprotein (mg/dL)	49 ± 10.5	51 ± 10.3	0.04
Low Density Lipoprotein (mg/dL)	89 ± 26.4	91 ± 29.7	0.27
Ratio TG/HDL	2.2 ± 1.6	1.9 ± 1.2	0.06
Control			
Total Cholesterol (mg/dL)	163 ± 29.0	162 ± 30.9	0.77
Triglycerides (mg/dl)	99 ± 42.6	94 ± 37.4	0.06
High Density Lipoprotein (mg/dL)	46 ± 7.7	46 ± 10.3	0.09
Low Density Lipoprotein (mg/dL)	97 ± 23.6	97 ± 26.8	0.69
Ratio TG/HDL	2.2 ± 1.1	2.1 ± 1.1	0.10

Student's-t test for dependent samples

More specifically, in obesity, changes occur in diverse points of the insulin signal transduction pathway. Such as reduction in the concentration and phosphorylation of the insulin receptors [19]. In many cases, this is explained by the higher level of subclinical inflammation in this population [20]. Hypertrophy of the adipose tissue stimulates the production of prof-inflammatory adipokines such as TNF-alfa and diminishes the production of anti-inflammatory substances such as adiponectin. This may consequently diminish insulin sensitivity, since TNF-alfa hinders, and adiponectin favors the action of insulin [21]. On the other hand, this process of physical exercise attenuates the sub-clinical inflammation, and improves the relations between the production of pro- and anti-inflammatory substances by the adipose tissue [9]. Although studies with only one exercise session have presented controversial results in this population [22, 23], thus acute reduction in subclinical inflammation is also a possible mechanism that explains the reduction in glycemia in the EG.

Other mechanisms independent of insulin may also explain the reduction in glycemia in the EG. The increase in bioavailability of chrome that occurs during and after exercise appears as one of the explanations. During exercise the increase in the need of glucose in muscle tissue stimulates the release of chrome that acts as adjuvant to insulin. Chrome potentiates the action of insulin, stimulating glucose absorption during and after exercise, increasing the fluidity of the cell membrane to facilitate insulin binding to its receptor [24]. This increase in the blood concentration of chrome may last for hours after exercise. The higher level of calcium release by the sarcoplasmic reticulum also favors glucose transport to the muscle cell [25]. The increase in calcium in the muscle cell cytoplasm initiates and facilitates activation of the molecules involved in the intracellular signaling cascade of glucose transport [25].

Table 5 Intergroup Analysis of Variation in Lipid Profile

	Control	Experimental	p
Δ Total Cholesterol	−1.0 (−6.5–3.5)	0.0 (−0.4–6.5)	0.32
Δ Triglycerides	−4.0 (−13.0–4.5)	−5.0 (−19.0–11.0)	0.80
Δ HDL	0.0 (−1.5–4.1)	0.6 (−1.0–2.8)	0.80
Δ LDL	−3.0 (−6.5–3.5)	0.0 (−4.0–6.5)	0.62
Δ TG/HDL	−0.1 (−0.3–0.1)	−0.1 (−0.4–0.1)	0.32

Median (Interquartile Interval); Mann–Whitney Test

A mechanism that is also independent of insulin is that of the AMPK enzyme (AMP-activated protein kinase). This enzyme stimulates glucose transport in the skeletal muscle. Its activation results in a reduction in the stocks of intracellular glucose. In the situation in which the AMP:ATP ratio increases, an increase in AMPK activity also occurs. This increase in AMPK activity in response to a need of generating ATP, particularly during but also after physical exercise, promotes the translocation of vessels containing Glut-4 [26]. This finally facilitates the influx of glucose into the muscle cell independently of insulin.

Lastly, another possibility may be associated with exercise-induced changes in hemodynamics. A single session of exercise is known to diminish sympathetic activity and increase muscle blood flow in the period after exercise. It is interesting to note that after a single session of exercise, sympathetic action diminishes and muscle vasodilatation increases. These and other hemodynamic changes may also contribute to increasing insulin sensitivity after exercise [27]. Moreover, physical exercise stimulated the production of endothelial nitric oxide synthase (eNOS), by means of shear stress on endothelial cells during exercise [28]. At the same time it inhibits a series of molecules that favor the production of Inducible nitric oxide synthase (iNOS). While the former is related to the higher level of vasodilatation of the active musculature [29], Inducible nitric oxide synthase is associated with insulin resistance [30]. Studies with rats have pointed out that a single session was capable of promoting a reduction in this enzyme and consequently increase insulin sensitivity.

Some of points of this study should be emphasized. The authors observed that although the study population was randomly divided, the CG presented higher insulin, Homa-IR and Homa-Beta values than the EG. Could this have influenced the results obtained? Individuals with a lower level of insulin sensitivity are known to present a higher concentration of plasma insulin in an attempt to maintain an adequate supply of glucose within the muscle and adipose cells. This was perceived when the HOMA-Beta values rose, demonstrating a higher level of insulin production by the beta-pancreatic cells.

Individuals who presented this condition had greater difficulty with metabolizing lipids, because when insulin binds to its muscle membrane receptors, it stimulates the action of lipoprotein lipases that play a fundamental role in the metabolism of triglycerides and plasma lipoproteins. However, as observed in Table 1, the fasting lipid profile values did not differ between the groups. Whereas, as observed in Table 4, in the EG there was a significant reduction in LDL and the Ratio TG/HDL, while no reduction was observed in the CG. Nevertheless, this reduction was not sufficient to show difference in the intergroup comparison.

Among the limitations of this study, the authors point out a single 12-h time interval of observation, and absence of caloric expenditure above 250Kcal for different comparisons. With regard to physical activity, it is known that factors such as time of observation and energy expenditure may influence the plasma limit response [31]. The authors observed that this study signals the responses to a caloric expenditure and in a specific time, and in this case no change was identified in the lipid response; which does not mean that these responses did not occur in later time intervals or in higher caloric expenditures. Comparisons of different protocols in the acute and sub-acute stage of physical exercise must be tested so that lipid responses to different energy expenditures and in different time intervals may be shown after the physical exercise session.

Conclusion

In overweight women, the sub-acute effect of low intensity physical exercise is capable of modifying the glycemic levels, not interfering in the lipid response and insulin resistance variables.

Acknowledgments

This work was supported by National Council for Scientific and Technological Development (CNPQ).

Funding

This work was supported by National Council for Scientific and Technological Development (CNPQ).

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Academic connection

This article is part of the doctoral thesis of Djeyne Silveira Wagmacker (Doctoral Program in Human Health), of the Bahian School of Medicine and Public Health, Salvador, BA – Brazil.

Authors' contribution

DSW, LEAR and AML conceived the idea and drafted the manuscript; ASF, JBM and SKAM worked on data collection; DSW and JP also performed the statistical analysis and AML and LEAR was also the supervisor. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The Research Ethics Committee of the Adventist Faculty of Bahia (CEP/FADBA), recognized by the National Commission for Research Ethics (CONEP/MS), analyzed the research protocol. Title: Influences of physical activity on the trans fatty acid profile in the serum of individuals with changes in body weight. CAAE: 34,017,514.5.0000.0042. Researcher Responsible: Djeyne Silveira Wagmacker. This project was APPROVED in its ethical and scientific aspects according to the Guidelines established in Resolution 466/12 of the National Health Council. DATE OF APPROVAL: 05/08/2014.

Consent for publication

The authors declare that the data met the criteria for authorship, as established by the International Committee of Medical Journal Editors and that the local Bioethics Committee of our center approved the protocol study and all patients provided informed consent.

Competing interests

The authors declare there is no pertinent conflict of interest.

Publisher's Note

Author details

[1]College Adventist Bahia, Cachoeira, Bahia, Brazil. [2]Bahian School of Medicine and Public Health, Science Development Foundation of Bahia, Bahia, Brazil. [3]Catholic University of Salvador, Salvador, Bahia, Brazil. [4]Present Address: Br 101, Km 197, cx postal 18, Salvador, Bahia, Brazil.

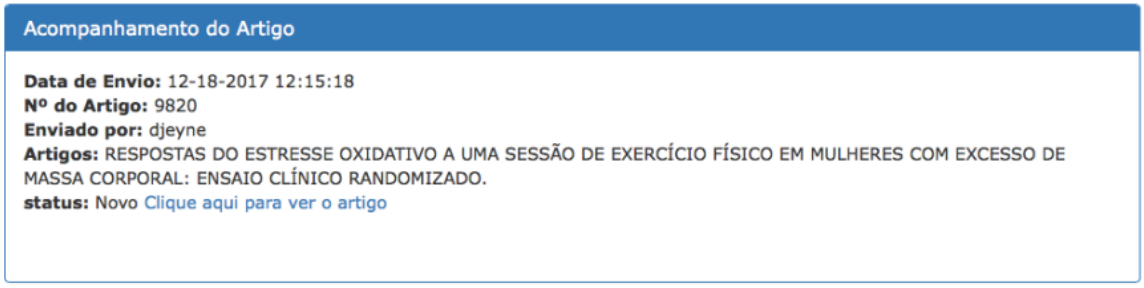
Received: 17 August 2017 Accepted: 26 October 2017
Published online: 19 December 2017

References

1. Petto J, Silveira DW, Santos AC, Seixas CR, Santo DG, Oliveira FT, et al. Postprandial lipemia and subclinical inflammation on active women taking oral contraceptive. Int J Cardiovasc Sci. 2015;28(3):215–23.
2. Magkos F, et al. Lipid metabolism response to a single, prolonged bout of endurance exercise in healthy young men. Am J Physiol Endocrinol Metab. 2006;290:E:355–E362.
3. Cambri LT, Decimo JP, Souza M, Oliveira FR, Gevaerd MS. Efeito agudo e crônico do exercício físico no perfil glicêmico e lipídico em diabéticos tipo 2. Motriz. 2007;13(4):238–48.
4. Bodel NG, Gillum T. 90 Minutes of Moderate-Intensity Exercise does not Attenuate Postprandial Triglycerides in Older Adults. Int J Exerc Sci. 2016; 9(5):677–84.
5. Thackray AE, Barrett LA, Tolfrey K. High-Intensity Running and Energy Restriction Reduce Postprandial Lipemia in Girls. Medicine & Science in Sports & Exercise. 2016;48(3):402–11.
6. Valdés PA, Ramos DC, Mehta R, Hernandez LM, Bautista IC, Mendez OP, et al. Factors associated with postprandial lipemia and apolipoprotein A-V levels in individuals with familial combined hyperlipidemia. BMC Endocr Disord. 2014;14:90.
7. Gill JM, Hardman AE. Exercise and postprandial lipid metabolism: an update on potential mechanisms and interactions with high-carbohydrate diets. J. Nutr. Biochem. 2003;14:122–32.
8. Petto J, Batista AKMS, Oliveira CS, Ladeia AMT. Efeito agudo do exercício físico na lipemia pós-prandial: revisão. Rev Bras Fisiologia Exercício. 2012;11:111–4.
9. Matsudo S, Araújo T, Marsudo V, Andrade D, Andrade E, Oliveira C, Braggion G. Questinário internacional de atividade física (IPAQ): estudo de validade e reprodutibilidade no Brasil. Rev bras ativ fis saúde. 2001;6(2):05–18.
10. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985;28:412–9.
11. Ep P, Mestek ML, Mahurin AJ. Postprandial triglyceride responses to aerobic exercise and extended-release niacin. Am J Clin Nutr. 2008;88(4):30–7.
12. Chen MJ, Fan X, Moe ST. Criterion-related validity of the Borg ratings of perceived exertion scale in healthy individuals: a meta-analysis. J Sports Sci. 2002;20:873–99.

13. Departamento De Aterosclerose Da Sociedade Brasileira De Cardiologia. IV Diretriz Brasileira Sobre Dislipidemias e Prevenção da aterosclerose. Arquivos Brasileitos de Cardiologia - Volume 88, Suplemento I, Abril 2007.
14. Ferguson MA, Alderson NL, Trost SG, Essig DA, Burke JR, Durstine JL. Effects of four different single exercises sessions on lipids, lipoproteins, and lipoprotein lipase. J Appl Physiol. 1998;85:1169–74.
15. Ferreira AP, Ferreira CB, Souza VC, Cordova COA, Silva CB, Nobrega OT, Franca NM. The influence of intense intermittent versus moderate continuous exercise on postprandial lipemia. Clinics. 2011;66(4):535–41.
16. Goodyear LJ, Kahn BB. Exercise, glucose transport and insulin sensitivity. Annu Rev Med. 1998;49:235–61.
17. Hayashi T, Wojtaszewski JF, Goodyear LJ. Exercise regulation of glucose transport in skeletal muscle. Am J Physiol. 1997;273(6):E1039–51.
18. Pauli JR, Ropelle ER, Cintra DE, Carvalho-filho MA, Moraes DSCT, et al. Acute physical exercise reverses S-nitrosation of the insulin receptor, insulin receptor substrate 1, and protein kinase B/Akt in dietary induce obese Wistar rats. J Physiol. 2008;586:659–71.
19. Dandona P, Aljada A, Bandyopadhyay A. Inflammation: the link between insulin resistance, obesity and diabetes. Trends immunology. 2004;25(1):4–7. 31
20. Waki H, Tontonoz P. Endocrine functions of adipose tissue. Annu Rev PatholMech Dis. 2007;2:31–56.
21. Petto J, Santos ACN, Motta MT, Filho RST, Santo DGCE, Ribas JLL, Ladeia AMT. Adiponectina: Caracterização, Ação Metabólica e Cardiovascular. Int J Cardiovasc Sci. 2015;28(5):424–32.
22. Fischer CP, Berntsen A, Perstrup LB, Eskildsen P, Pedersen BK. Plasma levels of interleukin-6 and C-reactive protein are associated with physical inactivity independent of obesity. Scand J Med Sci Sports. 2007;17(5):580–7.
23. Wagmacker DS, Souza KK, da Graça JV, Barreto GC, Macêdo PCS, Ferreira TF, et al. Acute effect of moderate intensity physical exercise on postprandial hyperlipaemia of individuals with central obesity. Health 4 2012; 1546–1550.
24. Evans GW, Bowman TD. Chromium picolinate increases membrane fluidity and rate of insulin internalization. J Inorg Biochem. 1992;46:243–50.
25. Holloszy JO, Constable SH, Young DA. Activation of glucose transport in muscle by exercise. Diabetes Metab Rev. 1986;1:409–24.
26. Hardie DG, Carling D. The AMP-activated protein kinase: fuel gauge of the mammalian cell? Eur J Biochem. 1997;246(2):259–73.
27. Bisquolo VA, Cardoso CG Jr, Ortega KC, Gusmao JL, Tinucci T, Negrao CE, et al. Previous exercise attenuates muscle sympathetic activity and increases blood flow during acute euglycemic hyperinsulinemia. J Appl Physiol. 2005; 98(3):866–71.
28. Gross SS, Wolin MS. Nitric oxide: pathophysiological mechanisms. Annu Rev Physiol. 1995;57:737–69.
29. Carvalho-Filho MA, Ueno M, Carvalheira JB, Velloso LA, Saad MJ. Targeted disruption of iNOS prevents LPS-induced S-nitrosation of IRbeta/IRS-1 and Akt and insulin resistance in muscle of mice. Am J Physiol Endocrinol Metab. 2006;291(3):E476–82.
30. Sugita H, Kaneki M, Tokunaga E, Sugita M, Koike C, Yasuhara S, et al. Inducible nitric oxide synthase plays a role in LPS-induced hyperglycemia and insulin resistance. Am J PhysiolEndocrinol Metab. 2002;282:E386–94.
31. Petto J, ARMS B, Oliveira CS, AMT L. Efeito agudo do exercício físico na lipemia pós-prandial. Revisão Rev Bras Fisiol Exercício. 2012;11:111–4.

5.3 Artigo 4 - Respostas do estresse oxidativo a uma sessão de exercício físico em mulheres com excesso de massa corporal: ensaio clínico randomizado. ***Arquivos Brasileiros de Cardiologia****. Em revisão*


Acompanhamento do Artigo

Data de Envio: 12-18-2017 12:15:18
Nº do Artigo: 9820
Enviado por: djeyne
Artigos: RESPOSTAS DO ESTRESSE OXIDATIVO A UMA SESSÃO DE EXERCÍCIO FÍSICO EM MULHERES COM EXCESSO DE MASSA CORPORAL: ENSAIO CLÍNICO RANDOMIZADO.
status: Novo Clique aqui para ver o artigo

COVER LETTER

One of the main mechanisms of injury caused by oxidative stress is the lipoperoxidation of the membrane, consequently causing alterations in its structure and permeability. Reactive oxygen species (ROS), which contribute to oxidative stress, may be related to a number of complications including atherosclerosis. Physical exercise has been pointed out as an important tool for the control of oxidative stress, but the results when evaluated the acute and chronic effects are still controversial.

The article titled Oxidative stress responses to a physical exercise session in women with excess body mass: Randomized Clinical Trial, developed by Djeyne Silveira Wagmacker, Jefferson Petto, Fabiano L. Silva, Jackeline Barbosa Moreira, Sindy Kerole Andrade Mota, Adriane B Kléin, Luiz Erlon Araujo Rodrigues and Ana Marice Ladeia aimed to test the hypothesis that a low to moderate intensity physical exercise session changes oxidative stress after 12 hours in women with increased body weight. The authors declare that the data meet the criteria of authorship established by the International Committee of Medical Journal Editors and that the local Bioethics Committee of our center approved the protocol study and all patients provided informed consent. The study was enrolled in the Clinical Track under protocol number NCT03170973.

The authors listed in the manuscript confirm that the work was not and will not be submitted to any other journal while it is under consideration in the Brazilian Archives of Cardiology and all have approved the submission of this version and assume full responsibility for the manuscript.

Author's contribution: DSW, FLS, JP, LEAR and AML conceived the idea and drafted the manuscript; JBM and SKAM worked on data collection; DSW and JP also performed the statistical analysis and AML, LEAR and ABK were also the supervisor.

This article represents honest work and the validity of its results can be certified. Furthermore, this article is part of Djeyne Silveira Wagmacker M.Sc Thesis for the Bahian School of Medicine and Public Health Post Graduate Course. And all authors declare no competing interest. This work was supported by National Council for Scientific and Technological Development (CNPq)

Sincerely

Djeyne Silveira Wagmacker

Jefferson Petto

Fabiano Leinchensering Silva

Jaqueline Barbosa Moreira

Sindy Kerole Andrade Mota

Adriane Belló Klein

Luiz Erlon Araújo Rodrigues

Ana Marice Ladeia

RESPOSTAS DO ESTRESSE OXIDATIVO A UMA SESSÃO DE EXERCÍCIO FÍSICO EM MULHERES COM EXCESSO DE MASSA CORPORAL: ENSAIO CLÍNICO RANDOMIZADO.

Wagmacker DS[1,2], Petto J[1,2], Silva FL[2], Moreira JB[1], Mota SKA[1], Klein AB[3], Rodrigues LEA[2], Ladeia AMT[1]

[1] FADBA - Faculdade Adventista da Bahia, [2] EBMSP- Escola Bahiana de Medicina e Saúde Pública, [3]UFRGS- Universidade Federal do Rio Grande do Sul.

RESUMO

Introdução: O estresse oxidativo está relacionado com o aparecimento de diversas doenças, incluindo as cardiovasculares. Fatores como o sedentarismo e obesidade predispõem ao aumento da formação de espécies reativas ao oxigênio. A atividade física têm sido considerada como um fator importante de redução do estresse oxidativo. **Objetivo:** Testar a hipótese de que uma sessão de exercício físico de baixa a moderada intensidade altera o stress oxidativo após 12 horas em mulheres com aumento do peso corporal. **Método:** Incluídas 30 mulheres, sedentárias, com excesso de peso corporal (IMC de 29±4,4kg/m^2), divididas aleatoriamente em dois grupos, controle e experimento. Após um jejum de 12 horas, as voluntárias fizeram uma primeira coleta de sangue. O grupo experimento foi submetido a uma sessão de exercício físico correspondendo a um gasto energético de 250Kcal com intensidade leve a moderada baseada na percepção de esforço de Borg, 12 horas após a primeira coleta de sangue. As voluntárias do grupo controle e experimento fizeram uma segunda coleta de sangue 24 horas após a primeira. Foram dosados triglicerídeos, colesterol total, TBARS, carbonilas e sulfidrilas. Foram utilizados teste t para amostras independentes e dependentes e adotando como nível de significância 5%. **Resultados:** O exercício físico modificou a resposta de peroxidação lipídica na análise intergrupo (controle = Δ 0,49 (-0,18 - 0,81) vs experimento= Δ-0,09 (-0,58 - 0,21)) (p= 0,02). Na análise intragrupo foi identificado um aumento do TBARS apenas no grupo controle (antes=1,97±0,65; depois=2,28±0,47; p=0,049). Não foram evidenciadas modificações para as sulfidrilas e

carbonilas nas análises intra e intergrupo. **Conclusão:** O efeito subagudo do exercício físico de baixa intensidade é capaz de modificar a resposta de peroxidação lipídica, não interferindo na peroxidação proteica e no fator antioxidante.

Palavras-chave: Obesidade; exercício ; TBARS; antioxidantes.

INTRODUÇÃO

O estresse oxidativo classicamente é conhecido como um desequilíbrio provocado entre a produção de espécies reativas de oxigênio (ROS) e a produção de fatores antioxidantes[1]. As espécies reativas do oxigênio em excesso dão início a uma cascata de reações moleculares gerando comprometimentos importantes como peroxidação lipídica, dano proteico e danos oxidativos em ácidos nucléicos[2]

Um dos principais mecanismos de lesão provocada pelo stresse oxidativo é a lipoperoxidação da membrana, acarretando consequentemente alterações na estrutura e na permeabilidade da mesma[3]. As espécies reativas de oxigênio (ROS), que contribuem para o estresse oxidativo, podem estar relacionadas à diversas complicações entre elas as doenças cardiovasculares e as relacionadas à obesidade, incluindo disfunção endotelial e aterosclerose[4]. A obesidade e o sedentarismo são considerados fatores contribuintes para a ocorrência de estresse oxidativo[5,6].

O exercício físico têm sido apontado como um fator positivo no controle do estresse oxidativo, porém os resultados ainda são contraditórios. Alguns estudos têm sinalizado que o exercício agudo pode induzir a um estado transitório de estresse oxidativo[7-8] outros estudos sugerem que o estresse oxidativo induzido pelo exercício possa promover a expressão da defesa antioxidante e as respostas adaptativas ao treinamento[9]. Alguns outros estudos têm sugerido que a atividade física pode reduzir o estresse oxidativo[10.]

Devido a grande variação de características relacionadas à pratica de exercício físico como intensidade, duração, modalidade, efeito agudo, subagudo e crônico do exercício, os resultados dos estudos apresentam resultados variados.

Quanto aos efeitos da intensidade do exercício sobre o estado redox os resultados ainda aparecem conflitantes[11]. Em alguns estudos, o exercício de alta intensidade têm sido sinalizado como responsável por aumentar o consumo de oxigênio e estimular mitocôndrias e linfócitos, resultando em maior produção de oxigênio reativo e espécies de nitrogênio (RONS)[12-13]. Outros estudos sugerem que o exercício de alta intensidade pode reduzir a produção de espécies reativas do oxigênio[14]. O exercício de baixa a moderada intensidade têm sido sugerido como positivo em alguns estudo na redução do estresse oxidativo[15] e em outros estudo seu efeito estressor parece estar presente.[16]

O tempo para que os efeitos sobre o estresse oxidativo apareçam após uma sessão de exercício físico também é um fator que carece de esclarecimentos. Em alguns trabalhos o efeito positivo sobre o estresse oxidativo foram percebidos somente 24h após o exercício[17], em outros estudos os efeitos sobre o estresse oxidativo já foram percebidos 12h após a sessão de exercício físico[18] .

A partir destes questionamentos e necessidades de maiores esclarecimentos quanto aos efeitos do exercício físico, o objetivo deste estudo foi testar as hipóteses de que uma sessão de exercício físico de baixa a moderada intensidade altera o estresse oxidativo após 12 horas em mulheres com excesso de peso.

MÉTODOS

Delineamento e população de estudo

Este ensaio clínico randomizado, registrado na ClinicalTrials.gov (NCT03170973), foi realizado com30 pacientes oriundos da Clínica escola da Faculdade Adventista da Bahia. A sua metologia já foi detalhadamente descrita em estudo publicado anteriormente na Lipids in Health and Disease. Abaixo, brevemente, os principais dados do protocolo.

Critérios de seleção

Os critérios de inclusão para participação neste estudo foram: Mulheres com índice de massa corporal (IMC) acima de 24,9kg/$m^{2,}$ com idade entre 18 a 30 anos, e sedentárias. O nível e sedentarismo foi

identificado a partir do Questionário Internacional de Atividade Física-versão longa. Foram excluídas mulheres que apresentassem doenças metabólicas, cardiovasculares, hipotireoidismo, doenças renais parenquimatosas ou diabetes mellitus, histórico de alcoolismo ou tabagismo, uso de hipolipemiantes, corticóides, diuréticos, beta-bloqueadores e anticoncepcionais.

Tamanho da amostra e randomização

A amostra foi de 30 pacientes, tendo sido selecionadas de forma aleatória 15 pacientes dentre 33 pacientes do grupo intervenção e 33 no grupo controle de ensaio clinico randomizado que incluiu no total de 66 pacientes. A randomização foi feita por um dos pesquisadores que não desempenharam um papel na intervenção ou avaliação laboratorial dos participantes.

Intervenções

Os pacientes em cada grupo foram submetidos à avaliação basal com coleta de sangue na veia antecubital após jejum de 12 horas para medição dos valores séricos de triglicerídeos, colesterol total, sulfidrilas, carbonilas e espécies reativas de ácido tiobarbitúrico (TBARs).

Passados 12 horas após a primeira coleta de sangue, as pacientes do grupo experimento realizaram uma sessão de exercício físico em esteira ergométrica. O exercício correspondeu a um gasto calórico de 250Kcal[19] e foi dividido nos tempos de: aquecimento (7 minutos), condicionamento e desaquecimento (5 minutos). O tempo de condicionamento foi o correspondente ao gasto energético de preconizado com intensidade leve baseada na percepção de esforço de Borg[20]. Para um melhor entendimento dessa escala foi realizado acoramento prévio ao dia do exercício habituando as voluntárias a responderem de forma adequada quando solicitado sobre a intensidade do exercício. Foi utilizado cardiofrequencímetro que mediu o gasto energético com base na massa corporal, sexo e idade da voluntária.

Após a sessão de exercício físico de baixa intensidade elas foram orientadas a retornar para casa e manter a sua dieta habitual. Vinte e quatro horas após a primeira coleta de sangue, as voluntárias retornaram ao

laboratório após um jejum de 12 horas e tiveram novamente amostras de sangue coletadas. Foram avaliadas as dieta dos dois dias anteriores ao exame de sangue através do recordatório alimentar de 24 horas.

As mulheres do grupo controle foram submetidas ao mesmo protocolo de coleta de dados do grupo experimento porém não realizaram o exercício 24h após a primeira coleta e foram orientadas a não realizarem atividade física nos dois dias prévios a coleta de sangue.

Ética da pesquisa

A proposta para esta pesquisa de tese foi apresentada ao Comitê de Ética da Faculdade Adventista da Bahia e aprovada sob número de protocolo 34017514.5.0000.0042. A participação neste estudo foi voluntária e os pacientes poderiam deixar o estudo em qualquer etapa. Todos os participantes foram informados sobre o objetivo e a natureza do estudo, e cada participante forneceu seu consentimento por escrito antes do estudo. Durante todo o estudo foram observadas as diretrizes sobre a pesquisa com seres humanos da Resolução 466/2012 do Conselho Nacional de Saúde.

Coleta de sangue e perfil metabólico

O sangue para análise foi coletado em dois momentos, dia basal e dia experimento, em ambos os grupos, após jejum de 12 horas. Para análise foram realizadas punções na veia antecubital, o sangue foi centrifugado, o soro foi aliquotado e congelado a –80 ºC para posterior análise. As análises séricas seguiram o seguinte protocolo;

As carbonilas - As amostras de sangue foram incubadas com 2,4 dinitrofenilhidrazina (DNPH 10 mmol/L) em solução de 2,5 mol/L HCl durante 1 h à temperatura ambiente, no escuro. As amostras foram submetidas a vortex a cada 15 min. Em seguida, adicionou-se 20% de solução de TCA (p / v) em amostras de tubos, deixou-se em gelo durante 10 min e centrifugou-se durante 5 min a 1000 g, para recolher precipitados de proteína. Outra lavagem foi realizada com 10% de TCA. O sedimento foi lavado 3 vezes com etanol: acetato de etilo (1: 1) (v / v). Os precipitados finais foram dissolvidos em solução de cloridrato de guanidina a 6 mol / L, deixados durante 10

minutos a 37 ° C e lidos a 360 nm.[21] Os resultados foram expressos como nmol / mg prot.

Para análise das sulfidrilas as amostras foram incubadas por 15 min, centrifugadas a 1800g por 15 min e lidas no comprimento de onda de 412nm. Foram utilizadas 100uL da amostra adicionados 300uL de tris 0,002mol/L, pH=8,2 e 20uL de solução de DTNB 0,01mol/L. O cálculo das sulfidrilas foi baseada na metodologia de Sedlak e Lindsau[22] e seguiu a fórmula: Sulfidrilas = (abs x diluição) / (13100x proteína).

Para avaliação da peroxidação lipídica foram mensuradas substâncias reativas ao ácido tiobárbiturico (TBARS). O protocolo utilizado seguiu a descrição de Buege e Aust[23]. Este método se baseia na reação de duas moléculas de ácido tiobarbitúrico com uma de malondialdeído (MDA), produzindo um complexo de coloração rósea que pode ser quantificado pela leitura em espectrofotômetro em um comprimento de onda de 532nm.

Foi adicionado a uma alíquota de 50 µL de plasma, 12,5 µL de SDS (8,1%), 93,75uL de ácido acético (20%), PH 3,5, 93,75 uL de ácido tiobarbitúrico (0,8%). Foi agitada e encubada em banho fervente por 1h. Foi resfriado em temperatura ambiente, centrifugado a 3500rpm por 10 min. O sobrenadante foi lido em espectofotômetro a tempertatura ambiente em um comprimento de onda de 532nm. A concentração dos TBARS foi determinada utilizando-se 1,56 x 105 x M-1mL-1 como coeficiente de extinção molar de MDA. Os valores foram expressos em ng de TBARs/mL de plasma.

Métodos estatísticos

A análise de dados foi realizada usando o software SPSS versão 24. Utilizamos o teste t de amostras independentes e teste t de amostras pareadas para analisarmos as diferenças inter e intragrupo respectivamente. A diferença estatística foi significante em $p < 0,05$. A normalidade dos dados foi testada utilizando o teste de Shapiro-Wilks.

RESULTADOS

Foram incluídas no estudo 30 mulheres jovens com idade média de 24,8±4,0 anos, com IMC de 29±4,1Kg/m^2, relação cintura quadril (RCQ) de 0,84±0,1. As mesmas apresentavam valores de triglicerídeos 100,8±55,5 e colesterol

de 161,7±31,2. Na Tabela 1 é possível observar as variáveis laboratoriais da amostra. Somente os valores de carbonilas diferiram entre os grupos, apresentando em maior valor no grupo experimento.

Tabela 1 – Aspectos clínicos, metabólicos e anti e pró-oxidantes de mulheres com aumento do peso corporal (n=30)

VARIÁVEIS	AMOSTRA TOTAL (n=30)	GC (n=15)	GE (n=15)	p
idade	24,8±4,0	25±4	25±3	0,97
IMC	29,4±4,1	29±4	29±3	0,86
RCQ	0,84±0,1	0,8±0,1	0,8±0,1	0,52
Triglicerídeos (mg/dL)	100,8±55,5	105±47	96±63	0,68
Colesterol total (mg/dL)	161,7±31,2	161±31	162±32	0,96
TBARS	1,9±0,48	1,97±0,62	1,87±0,34	0,59
Sulfidrilas	5,5±1,1	5,0±1,1	5,8±1,0	0,08
Carbonilas	16,1±5,8	12,0±5,7	19,4±3,4	0,01*

Na Tabela 2, observa-se a avaliação do percentual de consumo de carboidratos, gorduras e proteínas das pacientes com aumento do peso corporal. Não foram encontradas diferenças nas ingestas alimentares entre os grupos controle e experimento. O consumo de carboidrato foi o de apresentou a maior frequencia em ambos os grupos.

Tabela 2 – consumo alimentar das pacientes com aumento do peso corporal nos grupos

VARIÁVEIS	TOTAL (n=30)	GC (n=15)	GE (n=15)	p
Proteínas (%)	14,2±6,2	14,8±4,4	13,7±7,5	0,665
Carboidrato (%)	59,9±10,8	57,2±8,9	62,3±12,0	0,225
Lipídios (%)	25,7±8,5	27,8±8,1	23,8±8,8	0,225
Kcal totais	1873,3±831,3	1686,7±683,9	2035,0±933,5	0,277

Na tabela 3 observa-se a análise intragrupo da peroxidação lipídica (TBARS), peroxidação proteica (carbonilas) e do fator antioxidante (Sulfidrilas). Foi identificado aumento na peroxidação lipídica no grupo controle. No grupo experimento não foram identificadas mudanças nos valores de peroxidação lipídica, peroxidação proteica e no fator antioxidante.

Tabela 3 – Análise dos fatores antioxidante e pró-oxidantes antes e depois nos grupos controle e experimento, n=30.

	ANTES	DEPOIS	p
GE(n=15)			
TBARS	1,87±0,34	1,73±0,54	0,207
Sulfidrilas	5,86±1,08	5,52±1,51	0,465
Carbonilas	19,40±3,45	19,06±2,60	0,757
GC(n=15)			
TBARS	1,97±0,65	2,28±0,47	0,049*
Sulfidrilas	4,91±1,00	5,05±1,13	0,782
Carbonilas	12,20±5,34	12,30±6,05	0,939

Na tabela 4 quando comparadas as diferenças nos valores de peroxidação lipídica e proteica e nos valores do fator antioxidante, as diferenças ocorreram nos valores de TBARS entre os grupos, com redução para o grupo exercício.

Tabela 4 – Análise do **Δ** (valor depois- valor antes) dos valores antioxidante e pró-oxidantes nos grupos controle e experimento, (n=30).

	GC	GE	Valor de p
Δ TBARS	0,49 (- 0,18-0,81)	-0,09 (- 0,58-0,21)	0,032 *
Δ Sulfidrilas	0,04 (- 0,82-0,58)	-0,07 (- 1,67-1,03)	0,760
Δ Carbonilas	-1,50 (- 2,25-2,00)	0,00 (- 3,00-3,00)	0,978

DISCUSSÃO

O estresse oxidativo é medido pelo desequilíbrio entre fatores oxidantes e antioxidantes com um predomínio para o aumento dos fatores oxidantes. Esse desequilíbrio pode resultar em modificação oxidativa de DNA, lipídios e proteínas, desempenhando um papel fisiológico e patológico na saúde metabólica. Vias de identificação destas lesões relacionadas aos fatores oxidantes foram dosados neste estudo representados pelas espécies reativas de ácido tiobarbitúrico (TBARs)[24] como indicador de lipoperoxidação via malondialdeídos (MDA) e as carbonilas como indicador de peroxidação proteica.

A avaliação da peroxidação lipídica é difícil uma vez que a quantificação de radicais livres produzidos *in vivo*, têm uma meia vida curta e são altamente reativos[25]. Para que possa ser feita esta quantificação são utilizados métodos de mensuração de produtos de oxidação lipídica como indicadores indiretos da produção endógena de espécies Reativas de Oxigênio. Um dos produtos utilizados para dosar a intensidade da peroxidação lipídica é o malonaldeido (MDA). Este é formado pela decomposição dos hidroperóxidos lipídicos sendo caracterizado como um aldeído de cadeia curta e que pode ser medido pela reação com o ácido tiobarbitúrico (TBARs)[26].

O aumento de TBARs é um indicador indireto do aumento da peroxidação lipídica sendo, o marcador de lipoperoxidação mais comumente utilizado[27]. Pode-se observar que na análise intragrupo o grupo controle apresentou um aumento do TBARS enquanto que o grupo experimento manteve seus valores pós exercício semelhantes aos anteriores. Sabe-se que a inatividade física e a obesidade podem interferir aumentando o estresse oxidativo[28]. Segundo o estudo de Montes e col[26] as concentrações de TBARS de jejum correlacionaram-se com a circunferência da cintura e aumentaram em obesos em comparação com não obesos.

A manutenção dos valores de TBARS na análise intragrupo indica uma menor produção de fatores oxidantes no grupo exercício. Evidencias sugerem que a obesidade exacerbaria os níveis de estresse oxidativo após o exercício agudo, porém esta alteração pode ser provocada pelo tipo de exercício realizado. Neste estudo foi realizado exercício aeróbio de baixa

intensidade e os resultados evidenciaram redução nos valores de TBARs no grupo exercício. No estudo de Vicente e col[29] o tipo de exercício que provocou aumento dos fatores oxidante em população obesa foi o treinamento resistido com predomínio anaeróbio, não sendo evidenciadas alterações nos valores de TBARS para o grupo em que o exercício foi predominantemente aeróbico contínuo. Em nosso estudo, na análise intragrupo, o aumento do TBARS no grupo controle comparado a consequente manutenção dos valores de TBARS no grupo experimento sugerem que o exercício contínuo de baixa intensidade foi capaz de controlar a peroxidação lipídica em mulheres obesas.

Quando realizada a comparação das diferenças entre os grupos, observou-se que as medianas diferiram entre o grupo controle e experimento sendo identificadas por uma redução para o grupo experimento e um aumento no grupo controle. Dados de outro estudo sugerem que uma única sessão de exercício de baixa a intensidade indivíduos saudáveis atenua a resposta de estresse oxidativo[30]

Os grupo das Sulfidrilas são considerados os maiores e mais frequentes antioxidantes no plasma[31]. Quando comparados na análise intra e intergrupo não foram identificadas alterações significativas.

Alguns estudos sugerem que o exercício físico realizado de maneira aguda seria capaz de aumentar a produção endógena antioxidantes[32-33] porém tais benefícios parecem estar relacionados ao aumento do volume e da intensidade do treinamento, sendo que o exercício de alta intensidade provocaria maior estresse oxidativo porém com maior atividade antioxidante em comparação com o exercício de intensidade de baixa a moderada[34-36]. A capacidade estressora aguda provocada pelo exercício de alta intensidade poderia, em certa medida, regular a magnitude da adaptação protetora segundo algumas evidências[37]. A atividade física de baixa intensidade testada neste estudo evidenciou controle da atividade estressora com manutenção da atividade antioxidante.

CONCLUSÃO

De acordo com os resultados deste estudo em mulheres com excesso de peso, o efeito subagudo do exercício físico de baixa intensidade é capaz

de modificar a resposta de peroxidação lipídica, não interferindo na peroxidação proteica e no fator antioxidante.

POTENCIAL CONFLITO DE INTERESSES

Declaramos não haver conflito de interesses pertinentes.

FONTES DE FINANCIAMENTO

O presente estudo foi financiado pelo CNPq.

VINCULAÇÃO ACADÊMICA

Este artigo é parte da tese de doutorado de Djeyne Silveira Wagmacker pela Escola Bahiana de Medicina e Saúde Pública.

REFERENCIAS

(1) Hussein O, Shneider J, Rosenblat M, Aviram M. Valsartan therapy has additive anti-oxidative effect to that of fluvastatin therapy against low-density lipoprotein oxidation: studies in hypercholesterolemic and hypertensive patients. J Cardiovasc Pharmacol. 2002 Jul; 40(1):28-34.

(2) Betteridge DJ. What is oxidative stress? Metabolism. 2000; 49:3-8.

(3) Sinning C1, Westermann D1,2, Clemmensen P1,3 Oxidative stress in ischemia and reperfusion: current concepts, novel ideas and future perspectives. Biomark Med. 2017 Oct 17. doi: 10.2217/bmm-2017-0110. PMID: 29039206.

(4) Higdon JV, Frei B. Obesity and oxidative stress: a direct link to CVD? Arterioscler Thromb Vasc Biol, 2003;23:365-7.

(5) Montes-Nieto R, Insenser M, Murri M, Fernández-Durán E, Ojeda-Ojeda M, Martínez-García MÁ, Luque-Ramírez M, Escobar-Morreale HF.Plasma thiobarbituric acid reactive substances (TBARS) in young adults: Obesity increases fasting levels only in men whereas glucose ingestion, and not protein or lipid intake, increases postprandial concentrations regardless of sex and obesity. Mol Nutr Food Res. 2017 Jul 19. doi: 10.1002/mnfr.201700425. PMID: 28722287

(6) Tucker PS, Fisher-Wellman K, and Bloomer, RJ. Can exercise minimize postprandial oxidative stress in patients with type 2 diabetes? Curr. Diab. Rev. *2008;*4:309–319. doi: 10.2174/157339908786241160

(7) Fisher-Wellman K, Bell HK, and Bloomer, RJ. Oxidative stress and antioxidant defense mechanisms linked to exercise during cardiopulmonary and metabolic disorders. Oxid. Med. Cell. Longev. *2009* 2, 43–51. doi:10.4161/oxim.2.1.7732.

(8) Bentley DJ, Dank S, Coupland R, Midgley A, Spence I. Acute antioxidant supplementation improves endurance performance in trained athletes. Res Sports Med. 2012;20:1–12.

(9) Gomez-Cabrera MC, Salvador-Pascual A, Cabo H, Ferrando B, Viña J. Redox modulation of mitochondriogenesis in exercise. Doesantioxidantsupplementation blunt thebenefits of exercise training? Free Radic Biol Med. 2015; 86:37–46.

(10) Parker L, Shaw CS, Banting L, Levinger I, Hill KM, Andrew J, et al. Acute Low-Volume High-Intensity Interval Exercise and Continuous Moderate-Intensity Exercise Elicit a Similar Improvement in 24-h Glycemic Control in Overweight and Obese Adults. Front. Physiol., 2017; 661(7). https://doi.org/10.3389/fphys.2016.00661.

(11) Radak Z, Zhao Z, Koltai E, Ohno H, Atalay M. Oxygen consumption and usage during physical exercise: the balance between oxidative stress and ros-dependent adaptive signaling. Antioxid. Redox Signal. 2013*;*18:1208–1246. doi: 10.1089/ars.2011.4498.

(12) Jackson MJ, Pye D, Palomero J. Free radical biology in skeletal muscle the production of reactive oxygen and nitrogen species by skeletal muscle. J Appl Physiol. 2007; 102:1664–1670. doi: 10.1152/japplphysiol.01102.2006.

(13) Sachdev S, Davies KJ. Production, detection, and adaptive responses to free radicals in exercise. Free Radic Biol Med. 2008; 44(2):215–223. doi: 10.1016/j.freeradbiomed.2007.07.019.

(14) Gabriel B, Ratkevicius A, Gray P, Frenneaux MP, Gray SR. High-intensity exercise attenuates postprandial lipaemia and markers of oxidative stress. Clin. Sci. (Lond.). *2012;* 123, 313–321. doi: 10.1042/CS201060

(15) Lemos ET, Pinto R, Oliveira J, Garrido P, Sereno J, Mascarenhas-Melo F, Páscoa-Pinheiro J, Teixeira F, Reis F. Differential Effects of Acute (Extenuating) and Chronic (Training) Exercise on Inflammation and Oxidative Stress Status in an Animal Model of Type 2 Diabetes Mellitus. Mediators of Inflammation. 2011. doi:10.1155/2011/253061.

(16) Ji LL. Exercise, oxidative stress, and antioxidants. Am. J. Sports Med., 1996, 24: S20–S24.

(17) Takahashi M, Miyashita M, Park JH, Sakamoto S, Suzuki K. Effects of breaking sitting by standing and acute exercise on postprandial oxidative stress. Asian J. Sports Med. 2015. 6:e24902. doi: 10.5812/asjsm.24902

(18) Clean CM, Laughlin J, Burke G, Murphy MH, Trinick T, Duly E, et al. The effect of acute aerobic exercise on pulse wave velocity and oxidative stress following postprandial hypertriglyceridemia in healthy men. Eur. J. Appl. Physiol. 2007. 100, 225–234. doi: 10.1007/s00421-007-0422-y

(19) Plaisance EP, Mestek ML, Mahurin AJ, Postprandial triglyceride responses to aerobic exercise and extended-release niacin[1-3]. Am J Clin Nutr. 2008; 88(4):30-37.

(20) Chen MJ, Fan X, Moe ST. Criterion-related validity of the Borg ratings of perceived exertion scale in healthy individuals: a meta-analysis. J Sports Sci 2002; 20:873-99.

(21) Reznick AZ, Packer L, Oxidative damage to proteins: spectrophotometric method for carbonyl assay. Methods Enzymol.1994; 233:357-63.

(22) Sedlak J, Lindsau RH. Estimation of total protein bound and non protein sulphydryl groups in tissue with Ellman's reagentes. Biochen. 1968. 25: 192-205.

(23) Buege JA, Aust SD. Microssomal lipid peroxidation. Methods Enzymol. 1978;52: 302- 10.

(24) Dursun B, Dursun E, Suleymanlar G, Ozben B, Capraz I, Apaydin A, et al. Carotid artery intima- media thickness correlates with oxidative stress in chronic haemodialysis patients with accelerated atherosclerosis. Nephrol Dial Transplant. 2008. 23(5):1697–1703. https://doi.org/10.1093/ndt/gfm906

(25) Krüger RL, Farinha JB, Teixeira BC, Reischak-Oliveira A. Estresse oxidativo e a função endotelial: efeitos do exercício físico associado à lipemia pós-prandial. J Vasc Bras. 2015 Out.-Dez.; 14(4):328-340.

(26) Montes-Nieto R, Insenser M, Murri M, Fernández-Durán E, Ojeda-Ojeda M, Martínez-García MÁ, Luque-Ramírez M, Escobar-Morreale HF. Plasma thiobarbituric acid reactive substances (TBARS) in young adults: Obesity increases fasting levels only in men whereas glucose ingestion, and not protein or lipid intake, increases postprandial concentrations regardless of sex and obesity. Mol Nutr Food Res. 2017 Jul 19. doi: 10.1002/mnfr.201700425.

(27) Giacomini MM, Hahn S, Siqueira LO. Análise de correlação do perfil lipídico e dano oxidativo em pacientes diabéticos. Rev Ciênc Farm Básica Apl., 2013;34(2):251-255 ISSN 1808-4532

(28) Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free

radicals and antioxidants in normal physiological functions and human disease. Int. J. Biochem. Cell Biol. 2007; 39, 44–84. doi: 10.1016/j.biocel.2006.07.001

(29) Vincent HK, Morgan JW, Vincent KR. Obesity exacerbates oxidative stress levels after acute exercise. Med Sci Sports Exerc. 2004;36:772e779.

(30) Gabriel B, Ratkevicius A, Gray P, Frenneaux MP, Gray SR. High-intensity exercise attenuates postprandial lipaemia and markers of oxidative stress. Clin Sci 2012;123(5):313-21. http://dx.doi. org/10.1042/CS20110600. PMid:22435779.

(31) Pérez YG; Pérez LCG; Netto RCM; Lima DSN; Lima ES. *Malondialdeído e grupo sulfidrila como biomarcadores do estresse oxidativo em pacientes com lúpus eritematoso sistêmico.* Rev Bras Reumatol 2012;52(4):656-660

(32) Canale RE, Farney TM, McCarthy CG, Bloomer RJ. Influence of acute exercise of varying intensity and duration on postprandial oxidative stress. Eur J Appl Physiol. 2014;114(9):1913-24. http:// dx.doi.org/10.1007/s00421-014-2912-z. PMid:24907974.

(33) Berzosa C, CebrianI, Fuentes-BrotoL, et al. Acute exercise increases plasma total antioxidant status and antioxidant enzyme activities in untrained men. J Biomed Biotechnol. 2011;2011:540458. http:// dx.doi.org/10.1155/2011/540458. PMid:21436993.

(34) Schneider CD, Barp J, Ribeiro JR, Belló-Klein A, Oliveira AR, Oxidative Stress After Three Different Intensities of Running*Canadian Journal of Applied Physiology*, 2005; 30(6): 723-734, https://doi.org/10.1139/h05-1512005

(35) Fisher-Wellman K. Bloomer R. Acute exercise and oxidative stress: a 30 year history. *Dynamic Medicine* 2009, 8:1. doi:10.1186/1476-5918-8-1

(36) Lewan Parker, McGuckin, Anthony S. Leicht. Influence of exercise intensity on systemic oxidative stress and antioxidant capacity. 2014. 34(5)377–383. Doi: 10.1111/cpf.12108.

(37) Powers, S. K., Ji, L. L. and Leeuwenburgh, C. Exercise training-induced alterations in skeletal muscle antioxidant capacity: a brief review. Med. Sci. Sports Exercise. 1999; 31, 987–997.

5.4 Artigo 5 - Polyunsaturated fat is associated with subclinical inflammation in women with overweight. ***Motricidade***. *Em revisão.*

Unsaturated fat and subclinical inflammation

GORDURA POLIINSATURADA ESTÁ ASSOCIADA A INFLAMAÇÃO SUBCLÍNICA EM MULHERES COM EXCESSO DE PESO

POLYUNSATURATED FAT IS ASSOCIATED WITH SUBCLINICAL INFLAMMATION IN WOMEN WITH OVERWEIGHT

Original article

Acknowledgments:This work was supported by National Council for Scientific and Technological Development (CNPQ)

Financial support:This work was supported by National Council for Scientific and Technological Development (CNPQ)

GORDURA POLIINSATURADA ESTÁ ASSOCIADA A INFLAMAÇÃO SUBCLÍNICA EM MULHERES COM EXCESSO DE PESO

RESUMO

INTRODUÇÃO: O processo inflamatório é considerado fator central para desenvolvimento e progressão da aterosclerose. Diversos fatores podem influenciar o estado inflamatório como os padrões dietéticos e metabólicos. **OBJETIVO:** Verificar se existe associação entre inflamação subclínica e fatores alimentares e metabólicos em mulheres com excesso de peso. **MÉTODO:** Incluídas 66 mulheres com excesso de peso (IMC = 29±4,3kg/m^2), sedentárias, com idade de 24±4,1 anos. Dosados em jejum o perfil glicêmico, lipídico e proteína C reativa (PCR). O inquérito alimentar foi feito através do recordatório alimentar de 24h. Inflamação subclínica definida por PCR>3,0mg/L. Utilizados teste t, correlação de *Spearman* e regressão logística multivariada, com nível de significância p<0,05. **RESULTADOS:** Mulheres com inflamação vascular apresentaram valores maiores de glicemia 85±8,1 vs 83±7,9mg/dL (p=0,02) e IMC 32±5,6 vs 28±3,2kg/m^2 (p=0,02), menor consumo de gorduras poli-insaturadas 6±5,2 vs 10±8,1% (p=0,03) e de fibras 13±5,0 vs 20±13,8g/dia (p<0,01). Após regressão logística entre a PCR e as demais variáveis, permaneceram como fatores determinantes independentes o IMC (OR=1,2, IC95% 1,1-1,5) e o consumo de gorduras poli-insaturadas totais (OR=0,8 IC95% 0,7-0,8). **CONCLUSÃO:** Em mulheres com excesso de peso, o consumo de gorduras poli-insaturadas totais é fator protetor, enquanto que o do IMC é fator preditor independente para inflamação subclínica.

Palavras-chaves: Proteína C Reativa, Obesidade, Metabolismo, Nutrição.

POLYUNSATURATED FAT IS ASSOCIATED WITH SUBCLINICAL INFLAMMATION IN WOMEN WITH OVERWEIGHT

ABSTRACT

INTRODUCTION: The inflammation process is considered as a central factor with in the aspects that atherosclerosis. Several factors can affect the vascular inflammatory disease, such as dietary and metabolic patterns. **OBJECTIVE:** to verify if there is an association between subclinical inflammation and dietary and metabolic factors in women with overweight. **METHODS:** 66 women with overweight (BMI = 29±4,3kg/m^2), sedentary with the age of 24±4.1 years. Lipid profile, insulin and C-reactive protein (CRP) were dosed after fasting. The nutrition survey was made through the 24-hour recall. Subclinical inflammation defined by levels of CRP>3,0mg/L. It was utilized *t*-tests for independent samples, Spearman, multivariate logistic regression, as significance level p<0,05. **RESULTS:** Women with vascular inflammation present higher values of blood glucemic levels 85±8,1vs 83±7,9mg/dL (p=0,02) and BMI of 32±5,6 vs 28±3,2kg/m^2 (p=0,02), reduced intake of polyunsaturated fats 6±5,2 vs 10±8,1% (p=0,03) andfibers13±5,0 vs 20±13,8g/day (p<0,01). After the analysis of logistic regression, remained as independent decisive factors: the BMI (OR=1,2, IC95% 1,1-1,5) and the total intake of polyunsaturated fat (OR=0,8 IC95% 0,7-0,8). **CONCLUSION:** In women with overweight, the total polyunsaturated fats intakeis a protective factor, while increasing BMI is an independent predictor factor for the development of the subclinical inflammatory condition.

Keywords: C-Reactive Protein, Overweight, Metabolism, Nutrition.

INTRODUCTION

The inflammation process is considered a central factor within the aspects that determine atherosclerosis, both in relation to the development and to the progression. Several factors can affect the vascular inflammatory disease, including dietary and metabolic patterns (Francisco et al, 2006).

The C-reactive protein (CRP) is the inflammatory marker of the acute phase more used in the clinical practice, participating actively in the pathogenesis of atherosclerosis, besides being a predictor of cardiovascular events (Francisco et al., 2006; Silva and Lacerda, 2012).

The hypertrophy or hyperplasia of the adipose tissue has been related to an increase of the CRP concentrations. (Visser et al, 1999) Although considering the complexity of the physiological mechanisms involved with obesity, it is understood that adipocytes produce cytokines as Tumor Necrosis Factor alpha (TNF-α) and Interleukin 6 (IL-6). Once in the bloodstream, they stimulate the hepatocytes to produce acute phase proteins of inflammation, such as CRP. This protein can be increased in response to organic injuries even before appearing clinical manifestations of a concomitant cardiovascular disease (Brasil, et al 2007).

The consumption of lipids also can be a control factor or an inflammatory stimulus. (Lopez-Garcia et al., 2004; Niu et al., 2006) The polyunsaturated fats, for example, are considered precursors of eicosanoids and other anti-inflammatory mediators that could avoid several pathological conditions, including cardiovascular diseases (Roos, et al, 2009).

Nevertheless, until this moment, results about the actions of fatty acids in the inflammatory process are not yet conclusive (Saravanan et al, 2010).

Thus, more studies are necessary in order to clarify the role of diet and other risk factors in the induced inflammatory response in individuals with cardiovascular risk.

Therefore, the objective of this article is to verify if there is an association between subclinical inflammation and dietary and metabolic factors in women with overweight.

METHODS

DESIGN AND POPULATION STUDY

Analytical observational study with participants from Clínica Escola of the Adventist University at Bahia, in the city of Cachoeira, BA, Brazil. The samples were collected between September 2015 and May 2016.

All participants registered in the Physiotherapy service at Clínica Escola, who presented Body Mass Index (BMI) above 24.9kg/m^{2}were invited to participate in the study. Sixty-six volunteers fulfilled the inclusion criteria, which were: age between 18 and 30 years, BMI>24.9kg/m^2 and being sedentary. The sedentary was determinate based on the International Physical Activity Questionnaire – long version (Matsudo, 2001).

Were excluded individuals that presented cardiovascular disease, metabolic disorder, alcohol abuse or tobacco smoking history; use of hypolipidemic agents, corticosteroid, diuretic, beta-blockers, contraceptive pills, hypothyroidism, renal parenchymal disease or diabetes mellitus.

INSTRUMENTS

DATA COLLECTION PROTOCOL

The data collection protocol was divided into 4 parts: application of the standard questionnaire, physical examination, 24-hour recall, blood collection. The volunteers selected

answered initially to the standard questionnaire and were subjected to a physical examination. The physical examination included body mass index and stature. It was calculated Body Mass Index (BMI) from weight and height by Quetelet's equation: BMI= weight(kg)/height²(m).The waist circumference was measured with an inelastic tape at the midpoint between the iliac crest and the lastrib. The hip was measured at the greatest circumference of the gluteus.

24-HOUR RECALL

The patients were evaluated in relation to their diet in the day before the exam through a 24-hour recall. The 24-hour recall was made through an interview realized in the moment of blood collection, in which the volunteers answered about what they had consumed in the day before the interview – concerning the three main meals and the snacks. The same examiner applied this instrument, and in order to facilitate the answers, cooking measures were used. (Fisberg and Villar, 2002) The quantitative assessment of the diet was executed with the software *Avanutri Revolution*. In order to analyze data, micronutrients consumption (vitamins and minerals), cholesterol, saturated, total monounsaturated and polyunsaturated fat, total dietary fiber were considered using SBC parameters (Fisberg and Villar, 2002).

PROCEDURES

BLOOD COLLECTION AND METABOLIC PROFILE

The volunteers were subjected to blood collection after a 12 hours fasting. 5mL of blood in tubes with EDTA were collected and centrifuged at a speed of 3000 rotations/min.

The analysis of serum were made in the following way: the high-sensitivity CRP was made by Nephelometry. Glycemic and fasting total cholesterol levels were made through dry slide

method for blood glucose and dry for cholesterol. HDL cholesterol was measured by the cholesterol analysis method with an HDL *vitrus* KIT. Triglycerides (TG) were analyzed by dry slide method and the LDL was calculated by Friedwald Equation. (Sposito et al., 2007) The Homa-IR index of insulin resistance was calculated by the equation proposed by Mathews et al. (Matthews et al., 1985).

The individuals were classified as presenting subclinical inflammation when with CRP>3,0mg/L (Ghiringhello et al., 2006).

ETHICAL ASPECTS

The study was submitted to the Research Ethics Committee of the Adventist University at Bahia and was approved under the protocol l34017514.5.0000.0042. The guidelines about research with humans of the resolution 466/2012 of the Brazilian National Health Council were observed during the whole study.

STATISTICAL ANALYSIS

The data were previously analyzed in relation to symmetry by Kolmogorov-Smirnov test. The C-reactive protein (CRP) did not present normality criteria. The results were expressed by mean ± standard deviation or medians, and interquartile interval according to variable distribution. The significance level was defined by the value of $p<0.05$. Spearman was made between CRP and predictor metabolic and dietary factors. The variables that presented a statistical significance (blood sugar, insulin, HOMA, BMI, consumption of total polyunsaturated fats and fibers) were included in the logistic regression model. The calibration of the model was tested by Hosmer–Lemes how test and it was calibrated ($p= 0.07$). Data were analyzed using the software Statistical Package for the Social Science (SPSS) version 14.0.

169

170 **RESULTS**

171

172 It was included in the analysis 66 young women with age between 24 ± 3.6 years old, BMI
173 of 29 ± 4.3Kg/m^2, with lipid profile and with no alterations in the metabolic profile. The clinical
174 characteristics evaluated are described in Table 1.

175 In Table 2, It is depicted the mean consumption of vitamins, minerals, total polyunsaturated
176 fats and fibers in women with overweight.

177 As described in Table 3, women with vascular inflammation presented higher blood sugar
178 values(85 ± 8,1 vs 83 ± 7,9mg/dl) (p= 0.02) and BMI (32 ± 5.6; 28 ± 3.2kg/m^2) (p= 0.02), less
179 consumption of polyunsaturated fats(6 ± 5.2; 10 ± 8.1%) (p= 0.03), and fibers (13 ± 5.0 vs 20 ±
180 13.8g/day) (p= 0.01). There were not differences between HDL, LDL, total cholesterol and
181 triglycerides values (Table 3).

182 When the relation between metabolic and clinical aspects with CRP was analyzed, a
183 positive correlation between CRP and HOMA (r= 0.42; p= 0.01), insulin and inverse with fibers
184 was found. Association with other clinic-metabolic variables was not found (Figures 1, 2 and 3).

185 Multivariate logistic regression was made between CRP and the variables that presented a
186 statistical significance in the univariate analysis (blood sugar, insulin, HOMA, BMI,
187 polyunsaturated fats, and fibers). Remained as independent predictor variable for vascular
188 inflammation the variables BMI (*OR*= 1.27; *IC*= 1.079 – 1.505) and consumption of total
189 polyunsaturated fats (*OR*= 0.870; *IC* = 0.768 – 0.987), the last one presenting itself as a
190 predictor factor (Table 4).

191

192

DISCUSSION

Based on the results of this research it is possible to observe that women with overweight who consume more polyunsaturated fats present a lower subclinical inflammatory profile. From this, some points will be presented and discussed. It is observed in the population studied unaltered dietary profile according to Dietary Reference Intake (DRI) (Otten, et al, 2006) with vitamin deficiencies especially of vitamins A, D, B6, B12, C and E, as well as mineral deficiency of iron, zinc, and potassium, besides a high consumption of polyunsaturated fats and fibers. It is suggested that dietary patterns marked by low intake of high-fiber foods, by an increase in the consumption of trans fat, and by consumption of foods with high glycemic index could be the responsible for an activation of the immune system, which leads to over production of pro inflammatory mediators with concomitant reduction of anti-inflammatory mediators (Geraldo and Alfenas, 2008).

Studies have demonstrated that excess of body weight is associated with high CRP values. (Visser et al., 1999; Wu et al., 2010) In this study, it was possible to identify the subclinical inflammation in women with over weight, and, based on this value, they were characterized as with medium risk to cardiovascular disease. (Amezcua-Guerra et al, 2007) It is known that the inflammatory condition can be the responsible for the development, progression, and outcome of the atherosclerotic process, which is considered a precursor of cardiovascular diseases. (Hermann and Lerman, 2001) Despite that, there are several inflammatory biomarkers; the CRP has been the biomarker of the inflammatory process most largely studied (Kinlay and Egido, 2006; Calabrò , Golia, and Yeh, 2009).

The groups with and without inflammation were compared and it was possible to observe those volunteers who presented subclinical vascular inflammation, that is, CRP higher than

3mg/L, had higher glycemic values and BMI. The increase of CRP related to BMI is based, among other aspects, in the fact that adipose cells produce pro inflammatory cytokine that stimulates the production of the CRP by the liver – which explains the association between obesity and CRP. (Hotamisligil, et al., 1995) However, the elevation of plasma glicemic affects the oxidative stress initiating the inflammatory response, which leads to the concomitant CRP increase (Monnier et al., 2006).

It is also noticed a lower consumption of total polyunsaturated fats and fibers in the CRP group higher than 3mg/L. Different studies presented a relation between the consumption of fibers and an improvement of the inflammatory state, despite the consumption of fibers did not present itself as an independent variable to subclinical inflammation. (Delzenne and Cani, 2005; Ajani et al, 2004) The mechanisms through which the consumption of fibers reduces the concentration of CRP were not yet clarified. However, it is considered the possibility that the fiber slows the absorption of blood sugar and the modulation of cytokine, attenuating hyperglycemic, oxidative stress and favouring the response of the intestinal flora with the production of the anti-inflammatory cytokine.

Only the intake of polyunsaturated fats and BMI remained as determining variables for subclinical inflammation when logistic regression was made between variables that presented statistical significance in the univariate analysis. The relation between weight gain and the increase of inflammatory response is well explored in the literature. (Visser et al., 1999; Brasil et al., 2007) However, the relation between inflammation and consumption of polyunsaturated fats such as alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) presents conflicting results and still needs confirmation about its real effects (Rallidis et al., 2003; Lopez-Garcia et al., 2004; Madsen et al., 2001; Chan et al, 2002).

Besides the conflicting results, studies demonstrate that consumption of polyunsaturated fat is an important anti-inflammatory factor, which can act as a precursor of eicosanoids and other anti-inflammatory mediators, being able to improve several pathologic conditions including the cardiovascular (Lopez-Garcia et al., 2004; Niu et al., 2006).

Rallidis and cols observed a significant reduce in the CRP levels and IL-6 after ALA supplementation, whilelinolenic acid (LA) did not affect the concentrations of inflammatory markers.

Although observational studies suggest an inverse correlation between fish consumption or fish oil with high polyunsaturated fat (EPA and DHA) and the levels of inflammatory biomarkers,(Lopez-Garcia et al., 2004; Madsen et al., 2001) intervention studies did not confirm these effects (Chan et al., 2002).

CONCLUSION

According to results of this study, BMI and total intake of polyunsaturated are independent predictors of subclinical inflammation in women with an excess of body weight, the consumption of polyunsaturated fats being a protector mechanism, and the increase of BMI a trigger mechanism. The association with other variables, such as consumption of fibers, glycemic levels, insulin, HOMA suggests a multifactorial aspect in the genesis of the inflammatory response in women with overweight.

REFERENCES

Ajani, U. A., Ford, E. S., & Mokdad, A. H. (2004). Dietary fiber and C-reactive protein: findings from national health and nutrition examination survey data. The Journal of nutrition, 134(5), 1181-1185.

Amezcua-Guerra, L. M., Springall del Villar, R., & Bojalil Parra, R. (2007). Proteína C reactiva: aspectos cardiovasculares de una proteína de fase aguda. Archivos de cardiología de México, 77(1), 58-66.

Brasil, A. R., Norton, R. C., Rossetti, M. B., Leão, E.,& Mendes, R. P. (2007). C-Reactive protein as na indicator of low intensity inflammation in children and adolescentes with or without obesity. Jornal de pediatria, 83(5), 477-480.

Calabrò, P., Golia, E., & Yeh, E. T. (2009, June). CRP and the risk of atherosclerotic events. In Seminars in immunopathology (Vol. 31, No. 1, pp. 79-94). Springer-Verlag.

Chan, D. C., Watts, G. F., Barrett, P. H. R., Beilin, L. J., & Mori, T. A. (2002). Effect of atorvastatin and fish oil on plasma high-sensitivity C-reactive protein concentrations in individuals with visceral obesity. Clinical Chemistry, 48(6), 877-883.

De Roos, B., Mavrommatis, Y., & Brouwer, I. A. (2009). Long□chain n□3 polyunsaturated fatty acids: new insights into mechanisms relating to inflammation and coronary heart disease. British journal of pharmacology, 158(2), 413-428.

Delzenne, N. M., & Cani, P. D. (2005). A place for dietary fibre in the management of the metabolic syndrome. Current Opinion in Clinical Nutrition & Metabolic Care, 8(6), 636-640.

Fisberg, R. M., & Villar, B. S. (2002). Manual de receitas e medidas caseiras para cálculo de inquéritos alimentares: manual elaborado para auxiliar o processamento de dados de inquéritos alimentares. In Manual de receitas e medidas caseiras para cálculo de inquéritos alimentares: manual elaborado para auxiliar o processamento de dados de inquéritos alimentares. Signus.

Francisco, G., Hernández, C., & Simó, R. (2006). Serum markers of vascular inflammation in dyslipemia. Clinica chimica acta, 369(1), 1-16.

Geraldo, J. M., & Alfenas, R. D. C. (2008). Papel da dieta na prevenção e no controle da inflamação crônica: evidências atuais:[revisão]. Arq Bras Endocrinol Metabol, 951-967.

Ghiringhello, M. T., Vieira, J. G. H., Tachibana, T. T., Ferrer, C., Maciel, R., Amioka, P. H., ...& Reis, A. F. (2006). Distribution of HOMA-IR in Brazilian subjects with different body mass indexes. Arquivos Brasileiros de Endocrinologia & Metabologia, 50(3), 573-574.

Hermann J, Lerman A. (2001). The endothelium: dysfunction and beyond. J NuclCardiol. ; 8: 197-20

Hotamisligil, G. S., Arner, P., Caro, J. F., Atkinson, R. L., & Spiegelman, B. M. (1995). Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance. Journal of Clinical Investigation, 95(5), 2409.

Kinlay, S., & Egido, J. (2006). Inflammatory biomarkers in stable atherosclerosis. The American journal of cardiology, 98(11), S2-S8.

Lopez-Garcia, E., Schulze, M. B., Manson, J. E., Meigs, J. B., Albert, C. M., Rifai, N., ...& Hu, F. B. (2004). Consumption of (n-3) fatty acids is related to plasma biomarkers of inflammation and endothelial activation in women. The Journal of nutrition, 134(7), 1806-1811.

Madsen, T., Skou, H. A., Hansen, V. E., Fog, L., Christensen, J. H., Toft, E., & Schmidt, E. B. (2001). C-reactive protein, dietary n-3 fatty acids, and the extent of coronary artery disease. The American journal of cardiolo'gy, 88(10), 1139-1142.

Matsudo, S.; Araújo, T.; Marsudo, V.; Andrade, D.; Andrade, E.; Oliveira, l. C.; Braggion, G. (2001) Questinário internacional de atividade física(IPAQ): estudo de validade e reprodutibilidade no Brasil. Rev. Bras. Ativ. saude;6(2):05-18.

Matthews, D. R., Hosker, J. P., Rudenski, A. S., Naylor, B. A., Treacher, D. F., & Turner, R. C. (1985). Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia, 28(7), 412-419.

336 Monnier, L., Mas, E., Ginet, C., Michel, F., Villon, L., Cristol, J. P., & Colette, C. (2006).
337 Activation of oxidative stress by acute glucose fluctuations compared with sustained
338 chronic hyperglycemia in patients with type 2 diabetes. Jama, 295(14), 1681-1687.
339
340 Niu K, Hozawa A, Kuriyama S, Ohmori-Matsuda K, Shimazu T, Nakaya N, et al. 2006. Dietary
341 long-chain n-3 trans fats of marine origin and serum C-reactive protein concentrations are
342 associated in a population with a diet rich in marine products. Am J ClinNutr. ;84(1):223-
343 9.
344
345 Otten, J. J., Hellwig, J. P., & Meyers, L. D. (Eds.). (2006). Dietary reference intakes: the essential
346 guide to nutrient requirements. National Academies Press.
347
348 Rallidis, L. S., Paschos, G., Liakos, G. K., Velissaridou, A. H., Anastasiadis, G., & Zampelas, A.
349 (2003). Dietary α-linolenic acid decreases C-reactive protein, serum amyloid A and
350 interleukin-6 in dyslipidaemic patients. Atherosclerosis, 167(2), 237-242.
351
352 Santos R.D., Gagliardi A.C.M., Xavier H.T., Magnoni C.D., Cassani R., Lottenberg A.M. et al.
353 (20013) Sociedade Brasileira de Cardiologia. I Diretriz sobre o consumo de Gorduras e
354 Saúde Cardiovascular. Arq Bras Cardiol.;100(1Supl.3):1-40
355
356 Saravanan, P., Davidson, N. C., Schmidt, E. B., & Calder, P. C. (2010). Cardiovascular effects of
357 marine omega-3 fatty acids. The Lancet, 376(9740), 540-550.
358

Silva, D., & de Lacerda, A. P. (2012). Proteína C reativa de alta sensibilidade como biomarcador de risco na doença coronária. Revista Portuguesa de Cardiologia, 31(11), 733-745.

Sposito, A. C., Caramelli, B., Fonseca, F. A., Bertolami, M. C., Afiune Neto, A., Souza, A. D., ... & Carvalho, A. C. (2007). IV Diretriz brasileira sobre dislipidemias e prevenção da aterosclerose: Departamento de Aterosclerose da Sociedade Brasileira de Cardiologia. Arquivos Brasileiros de Cardiologia, 88, 2-19.

Visser, M., Bouter, L. M., McQuillan, G. M., Wener, M. H., & Harris, T. B. (1999). Elevated C-reactive protein levels in overweight and obese adults. Jama, 282(22), 2131-2135.

Wu, S. L., Li, J. F., Li, Y., Jin, C., Wang, L. Y., Ruan, C. Y., ...& Wang, J. L. (2010). The distribution and influential factors of serum high sensitivity C-reactive protein in general population. Zhonghua nei ke za zhi, 49(12), 1010-1014.

Table 1 – Clinical and anthropometric characteristics of the sample (n=66).

VARIABLES	VALUES
Age (years)	24±3,6 *
Body Mass Index (kg/m^2)	29±4,3*
Triglyceride (mg/dL)	94±43,5*
Total cholesterol (mg/dL)	162±32,3*
High-densitylipoprotein (mg/dL)	49±10,2*
Low-densitylipoprotein (mg/dL)	94±28,2*
Blood Sugar (mg/dL)	84±8,4*
Insulin (mcIU/mL)	10±4,9*
HOMA	2,4±1,2*
C-reactive protein basal level (mg/L)	1,1 (0,2 - 3,9)#
Inflammation	**n (%)**
CRP > 3,0 (mg/L)	46 (72)
CRP ≤ 3,0 (mg/L)	18 (28)

*Mean±SD#median (interquartileinterval).

396 **Table 2** – Dietary profile of women with alterations on body weight (n=66).
397

VARIABLES	VALUES
Vitamin A (RE/day)	262 (130 – 553)#
Vitamin D (mcg/day)	0,4 (0,1 - 1,1) #
Vitamin B1 (mg/day)	1±1,6*
Vitamin B2 (mg/day)	0,7±0,5*
Vitamin B5 (mg/day)	1,4 (0,9 - 2,2) #
Vitamin B6 (mg/day)	0,5 (0,3 – 1,2) #
Vitamin B12 (mg/day)	0,7 (0,1 – 1,8) #
Vitamin C (mg/day)	66 (18 – 213) #
Vitamin E (mg/day)	7,3 (2,8 – 15,2) #
Folic Acid (mcg/day)	60 (27 – 104) #
Calcium (mg/day)	346±262,5*
Phosphorus (mg/day)	604±314,5*
Iron (mg/day)	9,5 (6,7 – 13,8) #
Magnesium (mg/day)	124±69*
Zinc (mg/day)	4,6±3,5*
Selenium (mcg/day)	27 (13,9 – 56,6) #
Manganese (mg/day)	0,9 (0,6 – 1,6) #
Potassium (mg/day)	1119±620,6*
Sodium (mg/day)	2119±1400,7*
Cholesterol (mg/day)	156±152,5*
Saturated fat (%)	17±12,7*
Polyunsaturated fat (%)	8±7,4*
Monounsaturated fat (%)	10±7,3*
Fibers (g/dia)	17±12,3*

398 *Mean±SD#median (interquartileinterval).
399

400

401

402

403

Tabela 3 – Comparison of anthropometric, laboratory and dietaries aspects of the analyzed sample (n=66).

Variables	GVI Mean±SD/ Median (IQR)	GWVI Mean±SD/ Median (IQR)	P-value
Age (years)	24±2,9	24±3,9	0,93
BMI (Kg/m^2)	32±5,6	28±3,2	0,02*
Blood sugar (mg/dL)	84±8,1	83±7,9	0,02*
Insulin (mcIU/mL)	11±4,3	39±5,1	0,14
HOMA	2,7±1,2	2,2 ±1,2	0,11
Cholesterol (mg/dL)	154±26,9	163±33,6	0,29
Triglycerides (mg/dL)	96±40,9	92±45,5	0,71
HDL (mg/dL)	45,9 ±7,1	49,6±11,1	0,18
LDL (mg/dL)	88,4±24,7	95,1±29,1	0,39
Vitamin A (RE/day)	234 (115-625,7)	271 (150,2 – 564,8)	0,56
Vitamin D (mcg/day)	0,2(0,1 – 1,7)	0,4 (0,1 – 1,0)	0,11
Vitamin B1 (mg/day)	0,8 (0,4 - 1,2)	0,75 (0,5 – 1,3)	0,92
Vitamin B2 (mg/day)	0,7±0,6	0,6±0,3	0,52
Vitamin B5 (mg/day)	1,7 (0,8 – 3,3)	1,4 (0,9 – 2,1)	0,10
Vitamin B6 (mg/day)	0,4 (0,3 – 1,2)	0,6 (0,3 – 1,2)	0,60
Vitamin B12 (mg/day)	0,4 (0,0 – 2,1)	0,8 (0,2 – 1,6)	0,77
Vitamin C (mg/day)	47 (6,5 – 200,1)	87 (24,1 – 227,7)	0,97
Vitamin E (mg/day)	5 (2,5 – 8,8)	11 (2,9 – 16,2)	0,43
Folic Acid (mcg/day)	68±60,32	93±92,3	0,32
Calcium (mg/day)	392±321,7	328±242,4	0,39
Phosphoro (mg/day)	537±334,7	644±304,1	0,22
Iron (mg/day)	9,6 (6,4 – 11,3)	10,4 (6,7 – 15,3)	0,87
Magnesium (mg/day)	108±60,1	132±72,9	0,74
Selenium (mcg/day)	24 (13 – 55)	27 (14 – 58)	0,16
Sodium (mg/day)	2472±1698,2	2043±1246,2	0,27
Potassium (mg/day)	936±639,6	1216±604,5	0,10
Cholesterol (%)	95 (28 – 178)	126 (44 – 233)	0,53
Polyunsaturated fats (%)	6±5,2	10±8,1	0,03
Monounsaturated fats (%)	6,1 (4,0 – 12,9)	10,4 (6,1 – 14,6)	0,38
Fibers (g/dia)	12,7±5,0	19,76±13,8	<0,01

IQR – Interquartileinterval; GVI – Group with vascular inflammation; GWVI – Group without vascular inflammation

411 **Table4.**Predictor variables of vascular inflammation of the sample (n=66).
412

	OR*	CI95%	P-value
Blood Sugar (mg/dL)	0,931	0,832 – 1,041	0,21
Insulin (mcIU/mL)	0,898	0,589-1,369	0,61
HOMA	2,421	0,363 – 16,164	0,36
BMI (Kg/m^2)	1,275	1,079 - 1,505	<0,01
Polyunsaturated fats (%)	0,870	0,768 – 0,987	0,03
Fibers (g/day)	0,936	0,850 – 1,032	0,18

413 *Odds Ration
414

415

416

417

418

419

420

421

422

423

424

425

426

427

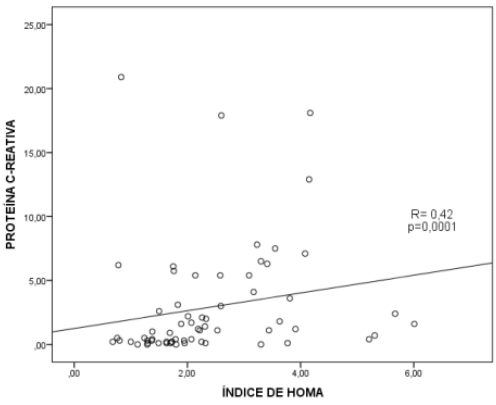


Figure 1 – Correlation between C-reactive Protein and HOMA

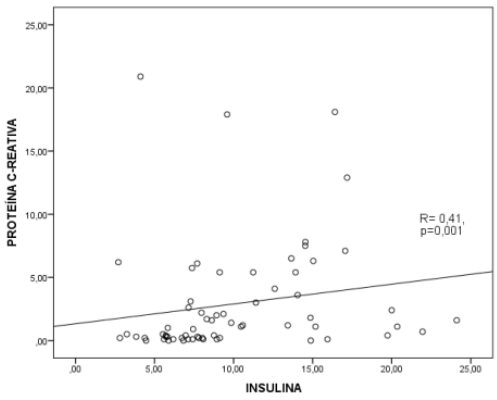


Figure 2 – Correlation between C-reactive Protein and insulin

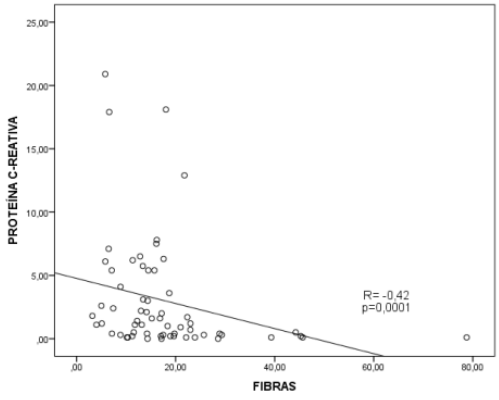


Figure 3 – Correlation between C-reactive protein and Fibers.

6 DISCUSSÃO

Um conjunto de produções científicas foram motivadas pelos objetivos dessa tese, contribuindo no desenvolvimento de um conhecimento amplo na relação tanto do efeito do exercício sobre os ácidos graxos, respostas metabólicas e de estresse oxidativo, como da relação dos ácidos graxos séricos e ingestão alimentar com indicadores de disfunções metabólicas e vasculares em mulheres com excesso de peso. Em resposta aos objetivos principais desta tese, foi avaliado o efeito agudo do exercício físico de baixa intensidade sobre diferentes aspectos metabólicos e de estresse oxidativo. Apesar da ampla utilização desse recurso terapêutico na prática clínica e em pesquisa nas últimas décadas, a ambos faltava uma avaliação metodológica rigorosa que pudesse contribuir para um posicionamento definitivo em relação aos seus reais efeitos. Adicionalmente, a relação do padrão de ingesta alimentar com alterações metabólicas e vasculares em mulheres com excesso de massa corporal. Ficou evidenciado que o consumo de gordura poliinsaturadas totais juntamente com o IMC são preditores independentes de inflamação subclínica em mulheres com excesso de peso. Os dados apresentados como resultado das investigações desta tese demonstraram originalidade, denotando importantes contribuições ao conhecimento científico.

Apesar das doenças cardiovasculares iniciarem um processo de declínio em países desenvolvidos, em países em desenvolvimento, como é o caso do Brasil, ainda estão entre as principais causas de morbidade, mortalidade e incapacidades[49]. Grande parte dos distúrbios cardiovasculares tem origem na aterosclerose, caracterizada por alterações na íntima, representadas por acúmulo de lipídeos, componentes do sangue, células, material intercelular e carboidratos[50].

Sabe-se que o processo aterosclerótico e outros fatores de risco cardiovasculares podem ser modulado por diferentes padrões dietéticos. As complicações secundárias ao aumento da ingestão de gordura, no entanto, não se restringem ao metabolismo lipídico; o tipo de gordura ingerida pode influenciar também em outros fatores de risco[51].

Os mecanismos relacionados às disfunções orgânicas provocadas pelo aumento do consumo de gorduras podem estar relacionados ao fato da sobrecarga lipídica induzir um aumento das lipoproteínas ricas em triglicerídeos - LpRT, redução do HDLc e à hiperinsulinemia. Esta condição metabólica leva à formação dos radicais livres, que, de acordo com a capacidade antioxidante (endógena e/ou exógena) presente, se diminuídas, determina estresse oxidativo. Os radicais livres estimulam os tecidos a secretarem citocinas

(TNF-α, IL-1 e IL-6), através dos macrófagos, estimulando assim a formação das moléculas de adesão. A geração de espécies reativas do oxigênio diminuem a biodisponibilidade do NO livre, resultando na menor vasodilatação dependente do endotélio e também na formação do peroxinitrito (ONOO), um potente e duradouro oxidante. Esses processos estão vinculados à gênese e progressão da lesão aterosclerótica[52-55].

No Brasil a população de indivíduos acima do peso têm aumentado consideravelmente nos últimos anos[56]. O acúmulo de tecido adiposo envolve um desequilíbrio entre energia consumida e energia gasta[57-58]. O excesso de energia, resultante deste desequilíbrio, é estocado nos adipócitos, que hipertrofiam e hiperplasiam[59-62]. Mais especificamente, na obesidade ocorrem alterações em diversos pontos da via de transdução do sinal da insulina, como redução na concentração e fosforilação dos receptores de insulina[63]. Isso, em muitos casos é explicado pela maior inflamação subclínica dessa população[64]. A hipertrofia do tecido adiposo estimula a produção de adipocinas pró-inflamatórias como o TNF-alfa e diminui a produção de substâncias anti-inflamatórias como a adiponectina. Isso pode por consequência diminuir a sensibilidade insulínica, já que, o TNF-alfa atrapalha e a adiponectina favorece a ação da insulina[65, 66].

A base para a prevenção de eventos cardiovasculares tem sido sugerida à partir do controle rigoroso dos fatores de risco. A prática de exercício regular vêm sendo estimulada na prática clínica, e vêm sendo apontada em diversos estudos como um mecanismo efetivo no controle de fatores de risco cardiovasculares, porém os resultados como o Ensaio clinico Randomizado HF: action[67] publicada em 2009, supreendem, mostrando diferentes resultados. A dificuldade na análises e interpretação destes estudos encontra-se no uso de diferentes protocolos de atividade física estabelecidos entre os estudos uma vez que a intensidade, duração e tipo de exercício interferem de maneira expressiva nos resultados, bem como as características clínicas e a capacidade funcional da população estudada[68].

O processo de captação e oxidação de ácidos graxos é significativamente importante, tanto em repouso como durante exercícios leves a moderados, os ácidos graxos são a fonte de substrato predominante para a re-síntese de ATP[3]. O metabolismo oxidativo que permite a obtenção de energia partir do ácidos graxos é de localização intramitocondrial. Para que o acil-CoA possa ser por ele utilizado é necessário vencer a impermeabilidade à acil-CoA da membrana externa e citoplasmática da mitocôndria. A enzima responsável por este transporte é a Carnitina-CoA aciltransferase (carnitina O-palmitil transferase). Esta enzima apresenta uma especificidade maior para o palmitil-CoA porém catalisa o transporte de ácidos graxos com tamanho de cadeia carbonada entre C4 a C18. Ácidos graxos maiores do que estes são

mais difíceis de serem transportados. Uma vez no interior da mitocôndria o acil-CoA poderá ser utilizado no metabolismo lipidolítico de Lynen.[69] Os ácidos graxos testados neste experimento, que possuem 9C (azeláico e Pelargônico) e 18C moléculas de carbono (oléico e elaídico), considerados ácidos graxos de cadeia media e longa teriam condições de serem metabolizados nas mitocôndria de maneira mais fácil para a produção de energia. Seria de se esperar que estes ácidos graxos apresentassem seus percetuais reduzidos após a atividade física.

Alguns estudos, realizados com outras populações e com protocolos diferentes, corroboram com nossos resultados e apontam que de forma aguda o exercício não é capaz de melhorar o perfil lipídico[69]. Um dos fatores que pode ter contribuído para a não modificação das lipoproteínas plasmáticas deste estudo pode estar associado ao baixo volume de exercícios. No estudo realizado por Ferguson et al.[70], foi investigada a correspondência entre o limiar energético e as possíveis mudanças nos níveis dos triglicerídeos e concentrações das lipoproteínas em homens treinados após quatro protocolos de exercício. Os protocolos foram realizados com gastos calóricos de 800, 1100, 1300 e 1500 kcal. Vinte e quatro horas após a realização das sessões, o nível de HDL elevou-se significativamente, nos exercícios de 1100, 1300 e 1500 kcal. Já a concentração de LDL diminuiu de forma significativa com um gasto de 1300 kcal e a de triglicerídeos com 800 kcal após uma única sessão de exercício. Ainda nesse estudo, foi possível observar aumento da atividade da lipase lipoproteica 24 horas após as sessões com gasto calórico acima de 1100 kcal, permanecendo elevada até 48 horas após a sessão de 1500 kcal, sendo que estas mudanças coincidiram com as alterações de HDL. Entretanto, sabe-se que para indivíduos obesos, intensidades e volumes elevados de treinamento físico estão associados à baixa adesão à prática de atividades físicas. Adicionalmente, a maior parte das mulheres investigadas neste estudo apresentavam perfil lipídico dentro da normalidade, tornando as modificações provocadas pelo exercício menos expressivas.

O protocolo utilizado neste estudo e descrito nos métodos em seção anterior, foi efetivo na redução da glicemia. O conhecimento de que o exercício aumenta a sensibilidade insulínica, tanto de forma aguda como crônica, serve de base para a explicação dos resultados obtidos nestes estudos[71]. Alguns são os efeitos promovidos pelo exercício que explicam esses resultados. Sabe-se que o exercício físico aumenta a fosforilação dos receptores de insulina, (IRS1 e 2) o que, por consequência facilita a ação da insulina[72]. Esse efeito ocorre durante o exercício e pode perdurar por até 16 horas após o exercício[73 -75].

A manutenção dos valores de TBARS na análise intragrupo observada nos resultados apresentados nas seções anteriores, sugere uma menor produção de fatores oxidantes no grupo exercício. Evidencias sugerem que a obesidade exacerbaria os níveis de estresse oxidativo após o exercício agudo, porém esta alteração pode ser provocada pelo tipo de exercício realizado, em nosso estudo foi trabalhado o exercício aeróbio de baixa intensidade e os resultados evidenciaram redução nos valores de TBARs no grupo exercício. No estudo de Vicente et al[76] a característica que provocou aumento dos fatores oxidante em população obesa foi a resistida com predomínio anaeróbio, não sendo evidenciadas alterações nos valores de TBARS para o grupo em que o exercício foi aeróbio contínuo. Em nosso estudo, na análise intragrupo, o aumento do TBARS no grupo controle comparado a consequente manutenção dos valores de TBARS no grupo experimento evidenciam que o exercício contínuo de baixa intensidade foi capaz de controlar a peroxidação lipídica em mulheres obesas.

Evidências sugerem que o exercício físico realizado de maneira aguda seria capaz de aumentar a produção endógena antioxidantes[77-78] porém tais benefícios parecem estar relacionados ao aumento do volume e da intensidade do treinamento. O exercício de alta intensidade provocaria maior estresse oxidativo porém com maior atividade antioxidante em comparação com o exercício de intensidade de baixa a moderada[79-80]. A capacidade estressora aguda provocada pelo exercício de alta intensidade poderia, em certa medida, regular a magnitude da adaptação protetora segundo algumas evidências[81]. A atividade física de baixa intensidade testada neste estudo evidenciou controle da atividade estressora com manutenção da atividade antioxidante.

Com base nas análises desta pesquisa foi possível observar que mulheres com excesso de peso, que ingerem mais gorduras poli-insaturadas apresentam menor perfil inflamatório subclínico. A despeito dos resultados conflitantes, estudos demonstram que o consumo de gordura poliinsaturada é um fator anti-inflamatório importante, podendo agir como precursor de icosanóides e outros mediadores anti-inflamatórios, sendo capaz de melhorar inúmeras condições patológicas, inclusive as cardiovasculares[82-84]. Rallidis et al[85] observaram redução significativa dos níveis de PCR e IL-6, após suplementação com Ácido alfa-linolênico (ALA), enquanto a suplementação com ácido linoléico (LA) não afetou as concentrações dos marcadores inflamatórios. Embora estudos observacionais sugiram correlação inversa entre o consumo de peixes ou óleos de peixe, com alto teor de gordura poliinsaturada (EPA e DHA) e o nível de biomarcadores da inflamação[86-87], estudos de intervenção não confirmaram estes efeitos[88].

7 CONCLUSÕES ESPECÍFICAS

Artigo 2 - O exercício físico de baixa intensidade não modifica, de maneira subaguda, os ácidos graxos de cadeia média e longa, em mulheres com excesso de peso.

Artigo 3 - Em mulheres com excesso de peso, o efeito subagudo do exercício físico de baixa intensidade é capaz de modificar a resposta glicêmica, não interferindo na resposta lipídica e inflamatória.

Artigo 4 - O efeito subagudo do exercício físico de baixa intensidade pode interferir na resposta de peroxidação lipídica, não interferindo na peroxidação proteica e no fator antioxidante.

Artigo 5 - O IMC e o consumo de gordura poliinsaturadas totais são preditores independentes de inflamação subclínica em mulheres com excesso de peso, sendo o consumo de gorduras poli-insaturadas um mecanismo protetor e o aumento do IMC um mecanismo desencadeador. Ainda, a associação com outras variáveis como consumo de fibras, os níveis de glicemia, insulina, índice de Homa sugerem um aspecto multifatorial na gênese da resposta inflamatória em mulheres com excesso de peso.

8 CONCLUSÃO GERAL

A resposta ao exercício físico de baixa intensidade é capaz de diminuir os valores glicêmicos e diminuir a peroxidação lipídica, porém não interfere no teor sérico dos ácidos graxos e de lipídios, na resposta antioxidante e na peroxidação proteica.

O consumo de gordura poliinsaturadas totais protege em relação à inflamação subclínica e o IMC aumenta o risco de inflamação subclínica em mulheres com excesso de peso.

9 MEMORIAL: DESCRIÇÃO E REFLEXÕES SOBRE O PROCESSO DE DOUTORADO E EXPERIÊNCIAS

Esta Tese de Doutorado inicia-se de uma inquietação acadêmico-científica sobre as reais respostas da atividade física frente à inúmeras evidências divergentes. Desde a minha graduação em fisioterapia tenho atuado na área de treinamento físico para pacientes com disfunções cardiovasculares e tenho me deparado com excelentes resultados clínicos frente a esta terapêutica. Porém as inquietações surgem pela dificuldade em encontrar comprovações sustentáveis para esta prática. Durante o período de mestrado me dediquei a estudar os efeitos agudos imediatos de uma sessão de exercício na lipemia pós-prandial. Os resultados mostraram que o para o exercício de moderada intensidade, não provocou mudanças no estado de lipemia pós-prandial em indivíduos obesos. Ao término do mestrado a inquietação persistiu na tentativa de entender em quais variáveis do metabolismo poderiam ser identificadas alterações com o protocolo utilizado. Ingressei em novembro de 2013 no programa de fluxo contínuo para o programa de doutorado. A perspectiva seria de continuar as análises da soroteca existente. Após 6 meses de início do programa de doutorado, me deparei com a realidade do descongelamento inesperado de toda a soroteca guardada, secundária a uma queda de temperatura do refrigerador.

Neste momento o desânimo e a frustação tornam-se aparentes, mas como diz a sagrada escritura “O choro pode durar uma noite mas a alegria vem pelo amanhecer”. Surge então a oportunidade de um novo recomeço, que apesar de árduo trouxe inúmeros aprendizados.

A partir destas preocupações uma nova temática evidência-se dentro da mesma vertente, exercício e metabolismo das gorduras em indivíduos com excesso de peso. O objetivo seria agora identificar os efeitos do exercício físico no metabolismo dos ácidos graxos (saturados, insaturados, cis e trans), na inflamação, no estresse oxidativo e correlações secundárias relacionadas ao perfil alimentar e metabólico desta população.

Um novo projeto foi escrito, e submetido ao comitê de ética com posterior aprovação. Uma metodologia mais robusta pode ser desenvolvida neste recomeço e optou-se por um Ensaio Clinico Randomizado que foi cadastrado no clinicaltrial.gov e registrado sob número de protocolo NCT 03170973. Este novo projeto foi também submetido a apreciação pelo CNPQ e contemplando para financiamento pelo edital Universal no ano de 2014, financiamento este que viabilizou um maior aprofundamento das investigações.

O ânimo e encorajamento novamente aflora e iniciou-se um novo processo de recrutamento de pacientes e início da coleta de dados que foi intensamente apoioado por

alunos PIBIC da FADBA e da Escola Bahiana de Medicina e Saúde Pública.

Após as coletas iniciou-se uma longa trajetória para preparo e leitura das amostras de sangue em cromatografia. Todo este processo se estendeu por um período de aproximadamente 32 meses até que os primeiros resultados pudessem ser obtidos. Os desafios superados envolveram desde treinamentos para operacionalização do equipamento, estudos e imersões com técnicos em cromatografia gasosa e preparo de equipamento envolvendo compra de materiais e ajustes do equipamento de modo que estivesse pronto para início das leituras.

O preparado das amostras de soro para injeção no cromatógrafo envolveu um longo e intenso aprendizado em técnicas laboratoriais. Todas as amostras e padrões precisaram ser transesterificadas para que pudessem ser lidas a partir da cromatografia gasosa. O processo de transesterificação envolveu duas etapas com tempo de intervalo entre elas de 7 a 14 dias por amostra, o que permitiu um maior tempo de imersão em técnicas laboratoriais. O processo de transesterificação foi realizado no laboratório de Neurociências da UFBA coordenado pela Dra Maria de Fátima Dias Costa sob a orientação do Dr. Luiz Erlon Rodrigues. Fiz todas as transesterificações das amostras com a ajuda de alunas do programa de PIBIC da Escola Bahiana e acompanhei as leituras dos padrões e das amostras que foram feitas com o apoio de um graduando em engenharia química financiado pela bolsa de apoio técnico do projeto.

As análises do estresse oxidativo foram feitas pelo laboratório de Fisiologia da UFRGS sob o acompanhamento da Dra. Adriane Bello-Klein. As amostras foram encaminhadas no gelo seco e foram dosados por alunas em programa de doutoramento nesta universidade.

Todas estas oportunidades de recomeços, parcerias, imersões e redes de contato estabelecidas neste programa de doutorado permitiram uma profunda e única oportunidade para o meu crescimento científico-acadêmico e imenso crescimento em técnicas de pesquisa. Término este memorial com uma frase de Martin Luter King apresentado no início desta tese que traduz muito bem a minha trajetória acadêmica:*"Eu não sou quem eu gostaria de ser; eu não sou quem eu poderia ser ainda, eu não sou quem eu deveria ser. Mas graças a Deus eu não sou mais quem eu era."*

.

10 PERSPECTIVAS FUTURAS

Pretendemos, a partir da soroteca existente deste estudo, continuar identificando o efeito do exercício físico em outras vertentes metabólicas, como no estado inflamatório, a partir das análises de citocinas. Os kits para as análises das citocinas TNF-alfa, IL-12, IL-10, IL-6, já foram adquiridos e serão analisadas pelo método Elisa no laboratório de imunologia da UFBA sob a supervisão do professor Hugo Bernardino.

Pretende-se aprofundar as correlações entre o perfil de ácidos graxos com alterações metabólicas e de estresse oxidativo a partir do banco de dados existente neste estudo.

REFERÊNCIAS

1. Moran AE, Forouzanfar MH, Roth GA, Mensah GA, Ezzati M, Murray CJ, et al. Temporal trends in ischemic heart disease mortality in 21 world regions, 1980 to 2010: the Global Burden of Disease 2010 study, Circulation, 2014; 129:1483e1492.

2. Finucane MM, Stevens GA, Cowan MJ, Danaei G, Lin JK, Paciorek CJ, et al. National, regional, and global trends in body-mass index since 1980: systematic analysis of health examination surveys and epidemiological studies with 960 country-years and 9.1 million participants. Lancet, 2011, 377:557e567.

3. Joint ESC Guidelines / Atherosclerosis, 2016; 252: 207e274.

4. Gottlieb MG, Bonardi G, Moriguchi EH. Fisiopatologia e aspectos inflamatórios da aterosclerose. *Scientia Medica*, 2005; *15*(3):203-207.

5. Shivappa N, Blair CK, Prizment AE, Jacobs DR, Steck SE, Hébert JR. Association between inflammatory potential of diet and mortality in the Iowa Women's Health study. European Journal of Nutrition, 2016;*55*(4):1491–1502.

6. Martin CA; Matshushita M, Souza NE. Ácidos graxos trans: implicações nutricionais e fontes na dieta. Rev. Nutr. [online]. 2004; 17(3), 351-359.

7. *Costa AGV, Bressan J, Sabarense CM.* Acidos graxos *trans*: Alimentos e efeitos na saúde. Archivos Latino Americanos de Nutricion (ALAN), 2006; 56(1):1-9.

8. Oomen CM, Ocke MC, Feskens EJ, Van Erp-Baart MA, Kok FJ, Kromhout D. Association between trans fatty acid intake and 10-year risk of coronary heart disease in the Zutphen Elderly Study: a prospective population-based study. Lancet. 2001;357(1): 746-571.

9. Perez-Matos MC, Morales-Alvarez MC, Mendivil CO, Lipids: A Suitable Therapeutic Target in Diabetic Neuropathy?. J Diabetes Res. 2017;2017:6943851.

10. Lopez-Garcia E, Schulze MB, Meigs JB, Manson JE, Rifai N, Stampfer MJ, et al. Consumption of trans fatty acids is related to plasma biomarkers of inflammation and endothelial dysfunction. J Nutr. 2005;135(1): 562-6.

11. Barres R, Yan J, Egan B, Treebak JT, Rasmussen M, Fritz T, et al. Acute exercise remodels promoter methylation in human skeletal muscle. Cell Metab, 2012; 15:405–411.

12. Egan B, Zierath JR. Exercise metabolism and the molecular regulation of skeletal muscle adaptation. Cell Metab, 2013; 17:162–184.

13. Petto J, Silveira DW, Santos AC, Seixas CR, Santo DG, Oliveira FT, et al. Postprandial lipemia and subclinical inflammation on active women taking oral contraceptive. Int J Cardiovasc Sci. 2015; 28(3):215-23.

14. Magkos, F. et al. “Lipid metabolism response to a single, prolonged bout of endurance exercise in healthy young men”. Am J Physiol Endocrinol Metab, 2006 290:E:355- E362.

15. Cambri LT, Decimo JP, Souza M, Oliveira FR, Gevaerd MS. Efeito agudo e crônico do exercício físico no perfil glicêmico e lipídico em diabéticos tipo 2. Motriz. 2007; 13(4):238-248

16. Bodel NG, Gillum T. 90 Minutes of Moderate-Intensity Exercise does not Attenuate Postprandial Triglycerides in Older Adults. Int J Exerc Sci. 2016; 9(5): 677-684.

17. Thackray AE, Barrett LA, Tolfrey K. High-Intensity Running and Energy Restriction Reduce Postprandial Lipemia in Girls. Medicine & Science in Sports & Exercise. 2016; 48(3): 402-411.

18. Jabbour G, Iancu HD, Paulin A, Lavoie JM, Lemoine-Morel S, Zouhal H. Effects of Acute Supramaximal Cycle Exercise on Plasma FFA Concentration in Obese Adolescent Boys. PLoS ONE, 2015; 10(6): e0129654.

19. Fisher-Wellman K, Bell H K, Bloomer RJ. Oxidative stress and antioxidant defense mechanisms linked to exercise during cardiopulmonary and metabolic disorders. Oxid. Med. Cell. Longev. 2009*;* 2, 43–51.

20. Bentley DJ, Dank S, Coupland R, Midgley A, Spence I. Acute antioxidant supplementation improves endurance performance in trained athletes. Res Sports Med. 2012; 20:1–12.

21. Gomez-Cabrera MC, Salvador-Pascual A, Cabo H, Ferrando B, Viña J. Redox modulation of mitochondriogenesis in exercise. Doesantioxidantsupplementation blunt thebenefits of exercise training? Free Radic Biol Med. 2015;86:37–46.

22. Parker L, Shaw CS, Banting L, Levinger I, Hill KM, McAinch AJ. et al. Acute Low-Volume High-Intensity Interval Exercise and Continuous Moderate-Intensity Exercise Elicit a Similar Improvement in 24-h Glycemic Control in Overweight and Obese Adults. Front. Physiol., 2017. 661(7).

23. Radak Z, Zhao Z, Koltai E, Ohno H, Atalay M. Oxygen consumption and usage during physical exercise: the balance between oxidative stress and ros-dependent adaptive signaling. Antioxid. Redox Signal. 2013; 18:1208–1246.

24. Jackson MJ, Pye D, Palomero J. Free radical biology in skeletal muscle the production of reactive oxygen and nitrogen species by skeletal muscle. J Appl Physiol. 2007;102:1664–1670.

25. Sachdev S, Davies KJ. Production, detection, and adaptive responses to free radicals in exercise. Free Radic Biol Med. 2008; 44(2): 215–223.

26. Gabriel B, Ratkevicius A, Gray P, Frenneaux MP, Gray SR. High-intensity exercise attenuates postprandial lipaemia and markers of oxidative stress. Clin. Sci. (Lond.).2012; 123: 313–321.

27. Lemos ET, Pinto R, Oliveira J, Garrido P, Sereno J, Mascarenhas-Melo F, et al. Differential Effects of Acute (Extenuating) and Chronic (Training) Exercise on Inflammation and Oxidative Stress Status in an Animal Model of Type 2 Diabetes Mellitus. Mediators of Inflammation. 2011.

28. Ji, L. L. Exercise, oxidative stress, and antioxidants. Am. J. Sports Med., 1996, 24: S20–S24.

29. Capó X, Martorell M, Sureda A, Riera J, Drobnic F, Tur, JA, et al. Effects of Almond- and Olive Oil-Based Docosahexaenoic- and Vitamin E-Enriched Beverage Dietary Supplementation on Inflammation Associated to Exercise and Age. Nutrients, 2016; *8*(10): 619.

30. Rocha-Rodrigues S, Rodríguez A, Gonçalves IO, Moreira A, Maciel E, Santos S, et al. Impact of physical exercise on visceral adipose tissue fatty acid profile and inflammation in response to a high-fat diet regimen.Int J Biochem Cell Biol. 2017; 87:114-124.

31. Matsudo S, Araújo T, Marsudo V, Andrade D, Andrade E, Oliveira LC, et al. Questinário internacional de atividade física(IPAQ): estudo de validade e reprodutibilidade no Brasil. Rev. bras. ativ. fís. saúde. 2001; 6(2):05-18.

32. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia, 1985; 28:412-9.

33. Prado, WL et al. Obesidade e adipocinas inflamatórias: implicações práticas para a prescrição de exercício. Rev Bras Med Esporte, 2009; 15(5): 378-383.

34. Reaven GM. Role of insulin resistance in human disease. **Diabetes 1988**; 37:1595-600.

35. Kalupahana NS, Moustaid-Moussa N, Claycombe KJ. Immunity as a link between obesity and insulin resistance. Molecular Aspects of Medicine. 2012; **33:** 26–34.

36. Jongh RT, Serné EH, Ijzerman RG, Vries G, Stehouwer CDA. Free fatty acid levels modulate microvascular function. Diabetes**.** 2004;53:2873-82.

37. Horowitz JF, Leone TC, Feng W, Kelly DP, Klein S. Effect of endurance training on lipid metabolism in women: a potential role for PPAR□□in the metabolic response to training. Am J Physiol Endocrinol Metab. 2000;279:E348-55.

38. Kiens, B. Skeletal muscle lipid metabolism in exercise and insulin resistence. Physiol. Rev. 2006; 86(1): 205-243, 2006.

39. Romijn, JA et al. Substrate metabolism during different exercise intensities in endurance-trained women. J. Appl. Physiol., 2000; 88(5):1707-1714.

40. Horowitz, JF, Klein S. Lipid metabolism during endurance exercise. Am. J. Clin. Nutr**.** 2000; 72(2):558S-563S.

41. Davis SN, Shavers C, Costa F. Gender-related differences in counterregulatory responses to antecedent hypoglycemia in normal humans. J Clin Endocrinol Metab. 2000; 85:2148–

2157.

42. Ziccardi P, Nappo F, Giugliano G, Esposito K, Marfella R, Cioffi M, et al. Reduction of inflammatory cytokine concentrations and improvement endothelial functions in obese women after weight loss over one year. Circulation. 2002;105:804-9.

43. Plaisance, EP. Mestek, ML. Mahurin, AJ. Postprandial triglyceride responses to aerobic exercise and extended-release niacin[1-3]. Am J Clin Nutr. 2008; 88(4):30-37.

44. Chen MJ, Fan X, Moe ST. Criterion-related validity of the Borg ratings of perceived exertion scale in healthy individuals: a meta-analysis. J Sports Sci 2002; 20:873-99.

45. Fisberg RM, Villar BS. Manual de receitas e medidas caseiras para cálculo de inquéritos alimentares. São Paulo: Signus, 2002.

46. Santos RD, Gagliardi ACM, Xavier HT, Magnoni CD, Cassani R, Lottenberg AM, et al. Sociedade Brasileira de Cardiologia. I Diretriz sobre o consumo de Gorduras e Saúde Cardiovascular. Arq Bras Cardiol. 2013; 100(1Supl.3):1-40.

47. Reznick AZ, Packer L, Oxidative damage to proteins: spectrophotometric method for carbonyl assay. Methods Enzymol.1994; 233:357-63.

48. Sedlak J, Lindsau RH. Estimation of total protein bound and non protein sulphydryl groups in tissue with Ellman's reagentes. Biochen. 1968; 25: 192-205.

49. Garelnabi M, Litvinov D, Parthasarathy S. Evaluation of a gas chromatography method for azelaic acid determination in selected biological samples. North American Journal of Medical Sciences. 2010; 2(9): 397-402.

50. Miyamoto S, Taylor SL, Barupal DK, et al. Systemic Metabolomic Changes in Blood Samples of Lung Cancer Patients Identified by Gas Chromatography Time-of-Flight Mass Spectrometry . Yeung PKF, ed. Metabolites. 2015;5(2):192-210.

51. Departamento De Aterosclerose Da Sociedade Brasileira De Cardiologia **.** IV Diretriz Brasileira Sobre Dislipidemias e Prevenção da aterosclerose**.** Arquivos Brasileitos de Cardiologia, 2007; 88(Suplemento I).

52. Sasaki EJ, Krause MP, Gama MPR. et al**.** Adiposidade abdominal nos níveis de proteína C-reativa em idosas**.** Arq Bras Cardiol. 2007; 89(4) : 231-236.

53. I Diretriz sobre o consumo de gorduras e saúde cardiovascular. Arq. Bras. Cardiol. [online]. 2013, 100(1): 1-40.

54. Dekker MJ, Graham TE, Ooi TC, et al. Exercise prior to fat ingestion lowers fasting and postprandial VLDL and decreases adipose tissue IL-6 and GIP receptor mRNA in hypertriacylglycerolemic men. Journal of Nutritional Biochemistry. 2010; 21:983-990.

55. Instituto Brasileiro de Geografia e Estatística (IBGE). [homepage na Internet]. Síntese de indicadores sociais 2002 [Citado 2006 abril 10]. Síntese de Indicadores Sociais confirma as desigualdades da sociedade brasileira. Disponível em: http://www.ibge.gov.br.

56. Signori LU et al. O papel da lipemia pós-prandial na gênese da aterosclerose: particularidades do diabetes mellitus. Arq Bras Endocrinol Metab, 2007; 51(2).

57. Matsudo SM, Matsudo VKR, Araújo T, et al. Nível de atividade física da população do Estado de São Paulo: análise de acordo com o gênero, idade, nível socioeconômico, distribuição geográ.ca e de conhecimento. Rev Bras Ciên Mov. 2000; 10(4):41-50.

58. Stapleton PA, Goodwill AG, James ME. Hypercholesterolemia and microvascular dysfunction: interventional strategies. J. Inflamm (Lond). 2010; 18;7:54.

59. Hermann J, Lerman A. The endothelium: dysfunction and beyond. J Nucl Cardiol. 2001; 8: 197-206

60. Nappo F, Esposito K, Cioffi M, Giugliano G, Molinari AM, Paolisso G, et al. Postprandial endothelial activation in healthy subjects and in type 2 diabetic patients: role of fat and carbohydrate meals. J Am Coll Cardiol, 2002; 39:1145-50.

61. Pinho RA, Araújo MC; Ghisi GLM, et al. Doença arterial coronariana, exercício físico e estresse oxidativo. Arq. Bras. Cardiol. 2010; 94(4).

62. Ridker PM, Glynn RJ, Hennekens CH. C-reactive protein adds to the predictive value of total and HDL cholesterol in determining risk of first myocardial infarction. Circulation. 1998; 97:2007-11.

63. Zhu S, Heshka S, Wang Z, et al. Combination of BMI and waist circumference, and health risk for identifying cardiovascular risk factors in whites. Obes Res. 2004;12:633-45.

64. Brasil AR, Norton RC, Rossetti MB, Leão E, Mendes RP. C-reactive protein as an indicator of low intensity inflammation in children and adolescents with and without obesity. J Pediatr (Rio J). 2007;83:477-80.

65. Amezcua–Guerra LM, del Villar RS, Parra RB. Proteína C reactiva: aspectos cardiovasculares de una proteína de fase aguda Arch. Cardiol. 2007; 7(1).

66. Dandona P, Aljada A, Bandyopadhyay A. Inflammation: the link between insulin resistance, obesity and diabetes. Trends immunology. 2004; 25(1):4-7.

67. Waki H, Tontonoz P. Endocrine functions of adipose tissue. Annu Rev Pathol Mech Dis. 2007; 2:31-56.

68. Petto J, Santos ACN, Motta MT, Filho RST, Santo DGCE, Ribas JLL, Ladeia AMT. Adiponectina: Caracterização, Ação Metabólica e Cardiovascular. Int J Cardiovasc Sci. 2015;28(5):424-432.

69. O'Connor CM, Whellan DJ, Lee KL, Keteyian SJ, Cooper LS, Ellis SJ, Piña IL. Efficacy and Safety of Exercise Training in Patients With Chronic Heart Failure: HF-ACTION Randomized Controlled Trial. *JAMA*:The Journal of the American Medical Association, 2009; *301*(14), 1439–1450.

70. Patton CM, Brandauer J, Weiss EP, et al. Hemostatic response to postprandial lipemia before and after exercise training. J. Appley Phisiology. 2006; 101:316-321.

71. Jayewardene AF, Mavros Y, Reeves A, Hancock DP, Gwinn T, Rooney KB. Interactions Between Fatty Acid Transport Proteins, Genes That Encode for Them, and Exercise: A Systematic Review. J Cell Physiol. 2016; 231(8):1671-87.

72. Harasim E, Kalinowska A, Stępek T, Chabowski A. The role of fatty-acid transport proteins (FAT/CD36, FABPpm, FATP) in lipid metabolism in skeletal muscles. Postepy Hig Med Dosw. (online), 2008; 62: 433-441.

73. Bodel NG, Gillum T. 90 Minutes of Moderate-Intensity Exercise does not Attenuate Postprandial Triglycerides in Older Adults. Int J Exerc Sci. 2016; 9(5): 677-684.

74. Ferguson MA, Alderson NL, Trost SG, Essig DA, Burke JR, Durstine JL. Effects of four different single exercises sessions on lipids, lipoproteins, and lipoprotein lipase. J Appl Physiol, 1998;85:1169-74.

75. Goodyear LJ, Kahn BB. Exercise, glucose transport and insulin sensitivity. Annu Rev Med, 1998;49: 235-61.

76. Hayashi T, Wojtaszewski JF, Goodyear LJ. Exercise regulation of glucose transport in skeletal muscle. Am J Physiol. 1997; 273(6): E1039-51.

77. Pauli JR, Ropelle ER, Cintra DE, Carvalho-filho MA, Moraes SCT, et al. Acute physical exercise reverses S-nitrosation of the insulin receptor, insulin receptor substrate 1, and protein kinase B/Akt in dietary induce obese Wistar rats. J Physiol. 2008; 586:659-71.

78. Vincent HK, Morgan JW, Vincent KR. Obesity exacerbates oxidative stress levels after acute exercise. Med Sci Sports Exerc. 2004; 36:772e779.

79. Canale RE, Farney TM, McCarthy CG, Bloomer RJ. Influence of acute exercise of varying intensity and duration on postprandial oxidative stress. Eur J Appl Physiol. 2014;114(9):1913-24.

80. Berzosa C, Cebrian I, Fuentes-Broto L, et al. Acute exercise increases plasma total antioxidant status and antioxidant enzyme activities in untrained men. J Biomed Biotechnol. 2011; 2011:540458.

81. Schneider CD, Barp J, Ribeiro JR, Belló-Klein A, Oliveira AR, Oxidative Stress After Three Different Intensities of Running. Canadian Journal of Applied Physiology, 2005, 30(6): 723-734.

82. Fisher-Wellman K. Bloomer R. Acute exercise and oxidative stress: a 30 year history. Dynamic Medicine, 2009, 8:1.

83. Lewan Parker, McGuckin, Anthony S. Leicht. Influence of exercise intensity on systemic oxidative stress and antioxidant capacity. 2014; 34(5):377–383.

84. Powers SK, Ji LL, Leeuwenburgh C. Exercise training-induced alterations in skeletal muscle antioxidant capacity: a brief review. Med. Sci. Sports Exercise. 1999; 31, 987–

997.

85. Lopez-Garcia E, Schulze MB, Manson JE, Meigs JB, Albert CM, Rifai N, et al. Consumption of (n-3) trans fats is related to plasma biomarkers of inflammation and endothelial activation in women. J Nutr. 2004;134(7):1806-11.

86. Niu K, Hozawa A, Kuriyama S, Ohmori-Matsuda K, Shimazu T, Nakaya N, et al. Dietary long-chain n-3 trans fats of marine origin and serum C-reactive protein concentrations are associated in a population with a diet rich in marine products. Am J Clin Nutr. 2006; 84(1):223-9.

87. Rallidis LS, Paschos G, Liakos GK, velissaridou AH, Anastasia-Dis G, Zampelas A. dietary alpha-linolenic acid decreases c- reactive protein, serum amyloid A and interleukin-6 in dyslipidaemic patients. Atherosclerosis. 2003; 167:237-42.

88. Lopez-garcia E, Schulze MB, Manson JE, Meigs JB, Albert cM, rifai n, et al. consumption of (n-3) trans fats is related to plasma biomarkers of inflammation and endothelial activation in women. J nutr. 2004; 134:1806-11.

89. Madsen T, Skou HA, Hansen VE, Fog L, Christensen JH, Toft E, et al. c-reactive protein, dietary n-3 trans fats, and the extent of coronary artery disease. Am J cardiol. 2001;88:1139-42.

90. Chan DC, Watts GF, Barrett PH, Beilin LJ, Mori TA. Effect of atorvastatin and fish oil on plasma high-sensitivity c-reactive protein concentrations in individuals with visceral obesity. clin chem. 2002; 48:877-83.

ANEXO

Anexo A – Carta de aprovação

COMITÊ DE ÉTICA EM PESQUISA - FACULDADE ADVENTISTA DA BAHIA
Mantida pela Instituição Adventista Nordeste Brasileira de Educação e Assistência Social
Credenciada pela Portaria nº 750, de 10/04/12, Publicada no D.O.U. em 13/04/12.

CEP

CARTA DE APROVAÇÃO

O Comitê de Ética em Pesquisa da Faculdade Adventista da Bahia – CEP/FADBA, reconhecido pela Comissão Nacional de Ética em Pesquisa – (CONEP/MS) analisou o protocolo de pesquisa:

Título: Influências da atividade física no perfil de ácidos graxos trans no soro de indivíduos com alterações de peso corporal.
CAAE: 34017514.5.0000.0042
Pesquisadora Responsável: Djeyne Silveira Wagmacker

Este projeto foi APROVADO em seus aspectos éticos e científicos de acordo com as Diretrizes estabelecidas na Resolução 466/12 do Conselho Nacional de Saúde.

DATA DA APROVAÇÃO: 05/08/2014

Cachoeira, 16 de agosto de 2017.

Wilma Raquel Barbosa Ribeiro
Coordenadora do Comitê de Ética em Pesquisa – CEP/FADBA

Estrada de Capoeiruçu - Rodovia BR 101, Km 197- Caixa Postal 18 - CEP: 44.300-000
Cachoeira/BA Fone (75) 3425-8055 Fax: (75) 3425-8101