UNIVERSIDADE ESTADUAL DE MONTES CLAROS

Deborah de Farias Lelis

Avaliação dos tecidos adiposos pericárdico e perivascular de indivíduos com doença arterial coronariana submetidos à cirurgia de revascularização miocárdica: um enfoque na obesidade

Montes Claros
2019

Deborah de Farias Lelis

Avaliação dos tecidos adiposos pericárdico e perivascular de indivíduos com doença arterial coronariana submetidos à cirurgia de revascularização miocárdica: um enfoque na obesidade

Dissertação apresentada ao Programa de Pós-Graduação em Ciências em Saúde da Universidade Estadual de Montes Claros – Unimontes, como parte das exigências para a obtenção do título de Mestre em Ciências da Saúde.

Área de Concentração: Mecanismos e Aspectos Clínicos das Doenças.

Orientador: Prof. Dr. Sérgio Henrique Sousa Santos.

Montes Claros
2019

Lelis, Deborah de Farias.
L541a Avaliação dos tecidos adiposos pericárdico e perivascular de indivíduos com doença arterial coronariana submetidos à cirurgia de revascularização miocárdica [manuscrito] : um enfoque na obesidade / Deborah de Farias Lelis. – 2019.
62f. : il.

Bibliografia: f. 38-41.
Dissertação (mestrado) - Universidade Estadual de Montes Claros - Unimontes,

Programa de Pós-Graduação em Ciências da Saúde/PPGCS, 2019.

Orientador: Prof. Dr. Sérgio Henrique Sousa Santos.

1. Obesidade. 2. Tecido adiposo. 3. Doença cardiovascular. 4. Sistema renina-angiotensina. I. Santos, Sérgio Henrique Sousa. II. Universidade Estadual de Montes Claros. III. Título. IV. Título: Um enfoque na obesidade.

Catalogação: Biblioteca Central Professor Antônio Jorge.

UNIVERSIDADE ESTADUAL DE MONTES CLAROS - UNIMONTES

Reitor: Antônio Alvimar Souza

Vice-reitora: Ilva Ruas de Abreu

Pró-reitor(a) de Pesquisa: Virgílio Mesquita Gomes

Coordenadoria de Acompanhamento de Projetos: Antônio Dimas Cardoso

Coordenadoria de Iniciação Científica: Sônia Ribeiro Arrudas

Coordenadoria de Inovação Tecnológica: Dario Alves de Oliveira

Pró-reitor(a) de Pós-Graduação: André Luiz Sena Guimarães

Coordenadoria de Pós-Graduação Lato-sensu: Augusto Guilherme Silveira Dias

Coordenadoria de Pós-Graduação Stricto-sensu: Maria de Fátima Rocha Maia

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS DA SAÚDE

Coordenador(a): Prof. Dr. Alfredo Maurício Batista de Paula

Subcoordenador(a): Prof. Dra. Marise Fagundes da Silveira

UNIVERSIDADE ESTADUAL DE MONTES CLAROS
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS DA SAÚDE

Unimontes

MESTRANDO(A): DÉBORAH DE FARIAS LELIS

TÍTULO DO TRABALHO: "Avaliação do perfil clínico e aspectos morfológicos e de expressão do sistema renina-angiotensina do tecido adiposo branco em pacientes eutróficos e com sobrepeso ou obesidade com doença arterial coronariana"

ÁREA DE CONCENTRAÇÃO: Mecanismos e Aspectos Clínicos das Doenças

LINHA DE PESQUISA: Clínica, Diagnóstico e Terapêutica das Doenças

BANCA (TITULARES)	ASSINATURAS
PROF. DR. SÉRGIO HENRIQUE SOUSA SANTOS- ORIENTADOR/PRESIDENTE	
PROF.ª DR.ª VALÉRIA MAFRA COTA	
PROF. DR. MARCELO PERIM BALDO	

BANCA (SUPLENTES)	ASSINATURAS
PROF. DR. JÚNIO COTA SILVA	
PROF. DR. ANDRÉ LUIZ SENA GUIMARÃES	

[X] **APROVADO** [ ] **REPROVADO**

Hospital Universitário Clemente Farias – HUCF
http://www.unimontes.br / ppgcs@unimontes.br
Telefone: (0xx38) 3224-8372 / Fax: (0xx38) 3224-8372
Av. Cula Mangabeira, 562, Santo Expedito, Montes Claros – MG, Brasil – Cep: 39401-001

Ao meu DEUS, porque tudo o que faço é para a honra e glória do Seu Santo nome.

AGRADECIMENTOS

Primeiramente agradeço a graça concedida por **Deus**, pois sem Ele este curso não teria sido concluído. Desejo que o resultado da minha defesa seja para engrandecer o nome d‘Ele e refletir a Sua glória em minha vida;

Ao amor da minha vida, **João Marcus**, que esteve comigo desde o início da jornada científica, ainda no primeiro período da graduação. Você foi uma das melhores escolhas que fiz na vida, agradeço a orientação, apoio, carinho, amor e encorajamento durante todo este tempo. Esta e muitas outras vitórias quero compartilhar com você;

Aos meus pais, **Sérgio** e **Denise**, os meus maiores apoiadores e incentivadores. Obrigada pelo esforço, sacrifícios e vontade de me permitir dedicar exclusivamente aos estudos. Esta vitória é também de vocês;

À minha irmã **Sarah**, obrigada por ter ouvido as minhas incansáveis reclamações e angústias ao longo deste período e pelas palavras de alívio. Você é e sempre será a minha fiel confidente;

Ao meu professor e orientador **Sérgio Henrique**, o meu mais profundo agradecimento por tudo o que aprendi sob a sua orientação. Muito obrigada pelas oportunidades de colaborações e participações em diversos trabalhos, o que me fez crescer pessoal, profissional e academicamente. Hoje saio uma pessoa diferente daquela que entrou no mestrado e sei o quão importante isso é para você;

Aos **professores e funcionários da secretaria do Programa de Pós-Graduação em Ciências da Saúde**, pelos ensinamentos, suporte, encorajamento e convivências quase que diárias;

Aos **componentes da banca examinadora, Marcelo Perim Baldo**, **Valéria Mafra, Júnio Cota e André Sena** que gentilmente aceitaram avaliar o meu trabalho, contribuindo para a melhoria deste;

A **Alanna**, que esteve presente desde a iniciação científica, obrigada pela amizade, ensinamentos, missão cupido e pelo carinho. Espero cultivar os nossos laços por toda a vida;

Às irmãs que ganhei no mestrado, **Jana, Amanda e Nati**, obrigada pelas noites regadas a vinho, os almoços na padaria, as compras e também pelo companheirismo durante todo este período. Sempre pude contar com vocês, eu serei eternamente grata por isso. Amizades que levarei para a vida;

A **Thaísa Crespo**, minha segunda mãe, amiga, médica, madrinha e companheira de projeto. O coração tem sido alvo dos nossos estudos e nos conecta cada vez mais. Conte sempre comigo, obrigada por TUDO;

A **Gislaine** e **Antônio Sérgio,** pela amizade e parceira nos trabalhos científicos. Vocês são praticamente família;

Aos **amigos do laboratório de pesquisa em saúde** e **colegas do mestrado**, sem vocês esta caminhada não teria sido fácil, obrigada pelo apoio e compartilhamento de alegrias e tristezas durante este tempo. Em especial cito Danilo Cangussu, Marcos Macedo, Lucas (Biol.), Lucas Figueiredo, Victor, Daniel, Thales, Jamille, Simone, Karla, Keila, Luís Paulo, Magda, Marcela, Sabrina, Rogério, Emisael, Dani (1 e 2), Handyara, Bere, Fábio, e Jaci, foi uma honra ter vocês comigo ao longo destes 08 anos.

À **CAPES, CNPq** e **FAPEMIG** pelo incentivo e fomento à pesquisa.

RESUMO

A doença arterial coronariana (DAC) constitui uma das principais causas de morbimortalidade no mundo, sendo desencadeada pela interação entre fatores ambientais e genéticos/moleculares. A obesidade destaca-se como o principal fator de risco para a DAC, regulando inúmeras vias e marcadores moleculares, entre eles o sistema renina-angiotensina (SRA). Buscando elucidar a relação existente entre DAC, obesidade e SRA, o objetivo deste estudo foi avaliar a histologia e a expressão de componentes do sistema renina-angiotensina em amostras de tecido adiposo pericárdico e perivascular de indivíduos eutróficos e com sobrepeso/obesidade e DAC. Foram obtidas no Biobanco de Material Biológico Humano do Norte de Minas Gerais (Biobanco Institucional – Universidade Estadual de Montes Claros/B-013), 19 amostras de tecido adiposo pericárdico e perivascular, sangue e dados clínicos associados, de pacientes submetidos à cirurgia de revascularização miocárdica que aceitaram doar as amostras ao biobanco referido. As amostras foram divididas em eutróficos e sobrepeso/obeso de acordo com o IMC. Foram avaliados dados antropométricos (Peso corporal, Índice de Massa Corporal, Circunferência de cintura, Circunferência de pescoço, Circunferência de quadril e Relação cintura-quadril) e clínicos (Obesidade, Diabetes, Dislipidemia, Hipertensão e Síndrome metabólica), bem como parâmetros bioquímicos (Colesterol total, Triglicérides, Lipoproteína de alta densidade (HDL), Lipoproteína de baixa densidade (LDL), Glicose e Proteína C-reativa ultrassensível (PCR ultrassensível). Foi avaliada também a área de adipócitos dos tecidos adiposos pericárdico e perivascular, após coloração de H&E, assim como a expressão de marcadores do SRA (Receptor de Angiotensina II tipo 1 – AT1 e Enzima conversora de angiotensina tipo 2 – ECA2) por PCR quantitativo em tempo real. As análises estatísticas foram realizadas nos softwares Graph Pad Prism e Statistical Package for Social Sciences. Os principais resultados encontrados mostraram que em nossas condições de investigação, a área dos adipócitos do tecido perivascular e os níveis bioquímicos de PCR ultrassensível estiveram aumentados nos pacientes com sobrepeso/obesidade quando comparados aos indivíduos eutróficos. Não observamos diferenças significativas nos dados clínicos avaliados entre indivíduos eutróficos e com sobrepeso ou obesidade e os parâmetros bioquímicos se mantiveram similares, o que pode ser explicado pelo uso de medicamentos antilipemiantes, anti-hipertensivos e/ou hipoglicemiantes utilizados pelos indivíduos. A área dos adipócitos esteve aumentada no tecido adiposo perivascular de indivíduos com sobrepeso ou obesidade em comparação aos indivíduos eutróficos. A expressão dos marcadores do SRA avaliados não diferiu entre grupos e/ou tecidos avaliados. Conclui-se que indivíduos com sobrepeso/obesidade e DAC apresentam um quadro inflamatório importante, dado evidenciado pelos níveis aumentados de PCR ultrassensível e área dos adipócitos do tecido adiposo perivascular aumentada, indicando hipertrofia dos adipócitos. A expressão dos marcadores do SRA parece similar entre grupos e tecidos. Em conjunto, os dados apresentados abrem novas perspectivas no entendimento do SRA e influência dos tecidos adiposos pericárdico e perivascular no contexto da obesidade e doença cardiovascular, encorajando futuras investigações que possam elucidar as lacunas aqui deixadas.

Palavras-chave: Obesidade. Tecido adiposo. Doença cardiovascular. Sistema renina-angiotensina.

ABSTRACT

Coronary artery disease (CAD) is one of the main causes of morbidity and mortality worldwide and is triggered by the interaction between environmental and genetic / molecular factors. Obesity stands out as the main risk factor for CAD, regulating several pathways and molecular markers, including the renin-angiotensin system (RAS). Aimimg to elucidate, the relationship between CAD, obesity and the RAS, the objective of this study was to evaluate the histology and expression of the renin-angiotensin system markers in the pericardial and perivascular adipose tissue samples from individuals with overweight/obesity with CAD. From the Bank of Human Biological Materials of North of Minas Gerais (Institutional Biobank - Montes Claros State University / B-013), 19 samples of pericardial and perivascular adipose tissue, blood and associated clinical data, were obtained from patients submitted to myocardial revascularization surgery who accepted to donate their biological sample to the aforementioned biobank. The samples were divided in eutrophic and overweight/obesity, according to the Body Mass Index. The following parameters were evaluated: Clinical data (Body Weight, Body Mass Index, Waist Circumference, Neck Circumference, Hip Circumference, Waist-to-hip ratio), biochemical parameters (total cholesterol, triglycerides, high-density lipoprotein (HDL), low-density lipoprotein (LDL), glucose and ultra-sensitive c-reactive protein (CRP ultra-sensitive)), as well as the adipocyte area of the pericardial and perivascular adipose tissues after H&E staining, and the expression of RAS componentes (Angiotensin II Receptor type 1 - AT1 and Angiotensin Converting Enzyme Type 2 - ACE2) by real - time quantitative PCR. The statistical analysis were performed in the Graph Pad Prism and Statistical Package for Social Sciences softwares. The main results showed that in our research conditions, the ultrasensitive PCR levels and adipocytes area in the perivascular adipose tissue from overweight/obese individuals were increased. No significant differences in the clinical data evaluated between eutrophic and overweight or obese subjects were seen. The expression of the RAS markers evaluated did not differ between the evaluated groups and / or tissues. It was concluded that the ultra-sensitive CRP and the adipocyte area in the perivascular adipose tissue are increased in the overweight/obesity state. The AT1 and ACE2 expression remained similar between groups and tissues. Therefore, the present study opens new perspectives in the understanding of the relationship among the RAS, obesity and cardiovascular diseases, evidencing the need for further studies to be performed.

Keywords: Obesity. Adipose tissue. Cardiovascular disease. Renin-angiotensin system.

LISTA DE ABREVIATURAS E SIGLAS

OMS	Organização Mundial da Saúde
IMC	Índice de Massa Corporal
TNF-α	Fator de Necrose Tumoral Alfa
IL-6	Interleucina-6
MCP-1	Proteína De Quimioatração De Monócitos
PAI-1	Inibidor Do Ativador De Plasminogênio Tipo 1
AGT	Angiotensinogênio
ECA	Enzima Conversora da Angiotensina
ECA2	Enzima Conversora da Angiotensina 2
Myf5	Fator Miogênico 5
UCP1	Proteína Desacopladora 1
DAC	Doença Arterial Coronariana
LDL-c	Colesterol de Baixa Densidade
Ang I	Angiotensina I
Ang II	Angiotensina II
Ang-(1–7)	Angiotensina (1–7)
Ang-(1-9)	Angiotensina-(1-9)
AT1	Receptor da Angiotensina II Tipo 1
AT2	Receptor da Angiotensina II Tipo 2
SRA	Sistema renina-angiotensina
PCR	Proteína C-Reativa
TAPv	Tecido adiposo perivascular
TAP	Tecido adiposo pericárdico

SUMÁRIO

1 INTRODUÇÃO

1.1 Obesidade

A obesidade é definida como o acúmulo excessivo de gordura corporal (tecido adiposo) (1). De acordo com a Organização Mundial de Saúde (OMS), indivíduos com Índice de Massa Corporal (IMC) maior ou igual a 24,9 Kg/m^2 são considerados sobrepesos, enquanto aqueles com IMC maior ou igual a 30 Kg/m^2 são considerados obesos. O IMC é calculado pela razão entre o peso corporal (quilogramas) e a $altura^2$ (metros). Inquéritos populacionais reforçam a existência de uma epidemia de obesidade em todo o mundo. Ainda segundo a OMS, em 2016, 39% dos adultos (com idade igual ou maior que 18 anos) apresentavam sobrepeso e 13% obesidade. Em números absolutos, mais de 1,5 bilhão de adultos apresenta sobrepeso e obesidade (2).

A obesidade é considerada uma doença crônica, causada pelo desequilíbrio entre consumo e dispêndio energético (3). Além disso, a grande maioria dos casos de obesidade está associada ao estilo de vida sedentário e ao consumo elevado de quilocalorias. De outro modo, a minoria dos casos está associada exclusivamente a fatores genéticos (4). No entanto, acredita-se que a interação entre fatores ambientais e genéticos fortalecem o desencadeamento de mecanismos etiopatogênicos associados à obesidade.

Ressalta-se ainda que a obesidade é fator de risco para diversas outras doenças, que se subdividem entre aquelas causadas pela hipertrofia de adipócitos, como o *diabetes mellitus*, doença hepática gordurosa não alcoólica, diversas doenças cardiovasculares, a colecistolitíase e o câncer; e aquelas causadas pela hiperproliferação dos adipócitos, como a osteoartrite e apneia obstrutiva do sono (1).

As comorbidades associadas à obesidade comprometem a qualidade de vida dos indivíduos que as possuem (5), incrementam os gastos com serviços de saúde e aumentam as taxas de morbimortalidade em diversos grupos de doenças (6). Ademais, diversos estudos identificaram os mecanismos fisiopatológicos da obesidade, atribuindo ao tecido adiposo branco os principais mecanismos reguladores de vias metabólicas e marcadores genéticos. Neste contexto, o tecido adiposo tem sido alvo majoritário, uma vez que, como órgão endócrino, influencia na modulação de diversas moléculas e na expressão de genes associados à suas comorbidades e que possuem impacto direto na homeostase metabólica (7).

1.2 Tecido adiposo

O tecido adiposo branco (TAB) foi considerado por muitos anos um órgão/tecido inerte que tinha como principais funções o isolamento térmico, a reserva energética, na forma de triacilgliceróis, e a proteção mecânica de estruturas externas e internas. No entanto, sabe-se hoje que este tecido é um dos maiores órgãos endócrinos do organismo e secreta mais de 600 tipos de biomoléculas ativas, o que faz dele um importante órgão/tecido com atividade secretora (8, 9). O TAB é o principal órgão de armazenamento de energia e possui, além dos adipócitos, outros tipos celulares contidos na porção do estroma-vascular, como as células imunes, sanguíneas, endoteliais, dentre outras (10-13). Pode-se citar como as principais funções fisiológicas do tecido adiposo: regulação do apetite, metabolismo da glicose e de lipídeos, reprodução, angiogênese, fibrinólise, homeostase do peso corporal, controle do tônus vascular, coagulação, imunidade, dentre outras (14-17).

As biomoléculas secretadas pelo tecido adiposo são comumente chamadas adipocinas e exercem funções locais e/ou sistêmicas, influenciando a homeostase metabólica (11). Dentre as principais adipocinas estudadas e descritas na literatura, podemos citar a leptina, adiponectina, resistina, fator de necrose tumoral alfa (TNFα), interleucina-6 (IL-6), proteína quimioatraente de monócitos-1 (MCP-1), inibidor do ativador de plasminogênio tipo 1 (PAI-1), componentes do sistema renina-angiotensina, como o angiotensinogênio (AGT), a angiotensina II (AngII), a enzima conversora de angiotensina (ECA), a enzima conversora de angiotensina II (ECA2) (11), confirmando o potencial endócrino deste órgão metabolicamente ativo.

Em mamíferos há dois tipos principais de tecido adiposo: o branco e o marrom (TAM). Os adipócitos destes dois tipos teciduais exibem importantes diferenças morfológicas, de localização e funcionais (18). O tecido adiposo marrom (TAM) se diferencia do adiposo branco, principalmente por ser derivado de progenitores miogênicos que expressam o fator miogênico 5 (Myf5), por ter adipócitos com depósito multilocular de gordura e alto número de mitocôndrias, sendo uma das suas principais funções a geração de calor/dispêndio energético via termogênese (19). O TAB, por sua vez, se origina de células tronco mesodérmicas e se subdivide em subcutâneo (formando uma camada subdérmica) e o visceral (circundando órgãos internos). O tecido adiposo branco subcutâneo ainda se subdivide de acordo com o sítio anatômico que envolve (ex. abdominal, glúteo e femoral, etc) enquanto o tecido adiposo branco visceral se divide em epicárdico, pericárdico, mesentérico, perivascular, retroperitoneal e gonadal (18).

A relação entre o tecido adiposo (em condições patológicas como a obesidade) e as doenças cardiovasculares (ex: aterosclerose) vem sendo amplamente discutido na literatura (20-23).

1.2.1 Tecido adiposo pericárdico

O tecido adiposo pericárdico (TAP), também chamado de paracárdico, mediastinal, intratorácido, dentre outras denominações, embora muitas vezes confundido e reconhecido como sinônimo do tecido adiposo epicárdico, se encontra entre o pericárdio visceral e parietal, adjacente ao saco pericárdico, estrutura formada pela fusão do pericárdio fibroso e a lâmina parietal do pericárdio seroso (24). Este tecido se origina do mesênquima torácico primitivo (25). Esse tipo de tecido adiposo é irrigado por diferentes fontes, por exemplo um ramo da artéria mamária interna e artérias pericardiofrênicas (ramos das artérias brônquicas, esofágicas e frênica superior) (26).

Análises comparativas mostraram que o tecido pericárdico apresenta adipócitos menores do que aqueles encontrados em depósitos subcutâneos de gordura e, ainda que alguns adipócitos apresentavam gotículas de gordura multiloculares, além da expressão aumentada da proteína desacopladora 1 (UCP1), características que o assemelham ao tecido adiposo marrom (27). A relevância destas características se faz no próprio metabolismo do tecido, uma vez que o processo termogênico característico do tecido adiposo marrom culmina em gasto energético, um objetivo comum no tratamento da obesidade e de suas comorbidades.

Estudos demonstraram correlação positiva entre o volume do TAP e os níveis plasmáticos de marcadores inflamatórios e ateroscleróticos. Além disso, observa-se associação entre o tecido adiposo pericárdico e doenças cardiovasculares, em quadros de obesidade/síndrome metabólica (28-34). Assim, discute-se que o TAP possa ser um importante preditor positivo ou fator de risco para doenças cardiovasculares (35).

O papel funcional do tecido adiposo pericárdico é ainda pouco entendido e controverso na literatura científica. Ainda existem muitas dúvidas acerca dos aspectos biomoleculares, anatômicos e funcionais do TAP, destacando a necessidade do incremento de estudos envolvendo este tecido, em especial em seres humanos.

1.2.2 Tecido adiposo perivascular

O tecido adiposo perivascular (TAPv) circunda vasos sanguíneos, independentemente da localização destes (36). Esse tecido origina-se na fase embrionária de diferenciação do folheto mesodérmico (37) e correlaciona-se positivamente com o volume de tecido adiposo intra-abdominal (visceral) (38, 39). O TAPv encontra-se em contato direto com a parede vascular (40) e foi descrito por apresentar forma irregular e tamanho reduzido quando comparado a outros depósitos de tecido adiposo (41).

O TAPv secreta diversas adipocinas com funções endócrinas e parácrinas e através da secreção de biomoléculas, comunica-se com os vasos que o circundam (36), exercendo influência sobre a resposta inflamatória e sobre o processo aterosclerótico (42). Este tecido tem efeitos na sensibilidade à insulina e no tônus muscular vascular (36). Tais efeitos são intensificados em quadros de disfunção metabólica e se explicam pelo contato direto deste tecido com os tecidos adjacentes; no caso do perivascular, a parede dos vasos.

Além disso, em condições de obesidade e aterosclerose, esse tecido apresenta infiltrado inflamatório, como reportado por diversos autores (43-45). Chatterje e colaboradores demonstraram que adipócitos do tecido adiposo perivascular têm a habilidade de invadir a camada adventícia dos vasos sanguíneos (46), interferindo no microambiente vascular. Ademais, o TAPv influencia na capacidade de vasodilatação e aumenta a taxa de recrutamento leucocitário vascular, induzindo disfunção endotelial (36, 47, 48).

1.3 Doença Arterial Coronariana

No Brasil, em 2012, as doenças cardiovasculares (DCV) foram responsáveis por aproximadamente 940.000 hospitalizações no Sistema Único de Saúde (SUS), impactando 18.3% dos custos em saúde (49). Dentre as DCVs, a doença arterial coronariana (DAC) é a principal causa de morte no mundo (50). O principal fator de risco para ela é a aterosclerose, a qual se inicia quando colesterol circulante, células inflamatórias e forças hemodinâmicas se combinam, rompendo os mecanismos homeostáticos vasculares e induzindo quadro hipoxêmico tecidual (51). As moléculas de colesterol plasmática de baixa densidade (LDL-c) e leucócitos aderem-se às paredes de artérias, induzindo lesão endotelial (50, 52, 53).

Partículas de LDL-c circulantes, ao invadirem as paredes arteriais se oxidam e se acumulam na parte íntima dos vasos (camada mais interna do vaso sanguíneo), induzindo ativação de células endoteliais e moléculas de adesão. Estas modificações, por sua vez, recrutam células inflamatórias, como macrófagos, que internalizam moléculas de colesterol e se tornam células espumosas “foam cells”, que inicializam a formação da placa de ateroma

(50). Outras moléculas de lipídios, como triglicérides e ácidos graxos livres, ativam outras vias inflamatórias alternativas, também contribuindo para o processo aterosclerótico (50). A figura 1 ilustra com mais detalhes diversas moléculas e células envolvidas no processo de dano vascular/aterosclerose.

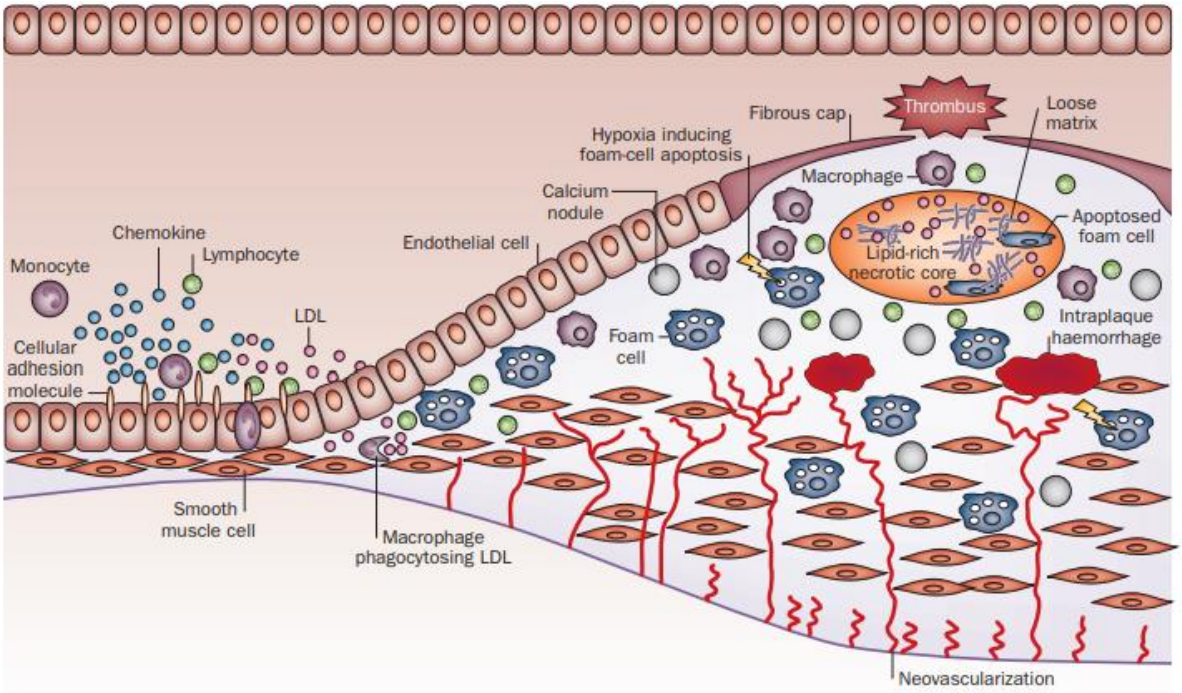

Figura 1. Desenvolvimento da placa de ateroma. A ativação endotelial leva à produção de quimiocinas e expressão de marcadores na superfície de células endoteliais, consequentemente causando a migração e adesão de células imunes no endotélio. A permeabilidade aumentada do endotélio permite que células e moléculas de LDL atravessem a parede dos vasos, favorecendo, então, a formação de células *foam* (macrófagos após absorção de LDL-c). A formação de células *foam* e proliferação de células do músculo liso levam a um espessamento localizado da parede vascular, iniciando a formação da placa. Células *foam* sofrem apoptose em condições de hipóxia e estresse oxidativo, levando à formação de um núcleo necrótico rico em lipídios, que por sua vez recebe deposição de cálcio o qual contribui para a formação de uma capa fibrosa. A progressão da placa leva ao estreitamento do lúmen e remodelamento da parede vascular. Fonte: Sarah Skeoch & Ian N. Bruce, 2015 (54).

Dentre os principais fatores de risco para a DAC, podemos citar dietas hipercalóricas, tabagismo, alcoolismo, sedentarismo, hipertensão arterial, dislipidemia, hiperglicemia, obesidade, dentre outros (52). A CAD pode levar a: 1) angina estável (crônica): que normalmente ocorre em situações de stress/esforço físico, caracterizada por oclusão limitante da perfusão sem ruptura da placa; 2) síndrome coronariana aguda (angina instável), que ocorre em condições usuais, com ruptura da placa e oclusão vascular transitória ou incompleta e; 3) infarto agudo do miocárdio (com e sem supra do segmento ST), em que a placa se rompe causando oclusão vascular total e dano tecidual (50).

Pacientes obesos apresentam risco elevado para o desenvolvimento de DAC. Esta associação é explicada principalmente pela capacidade endócrina do tecido adiposo branco que, ao secretar moléculas bioativas, especialmente pró-inflamatórias, contribui para a disfunção endotelial e consequentemente aterosclerose (Figura 2). Um estudo realizado por McGill e colaboradores, que incluiu necrópsia de mais de 3.000 indivíduos jovens, reportou associação positiva entre Índice de Massa Corporal (IMC) e lesões ateroscleróticas (53, 55).

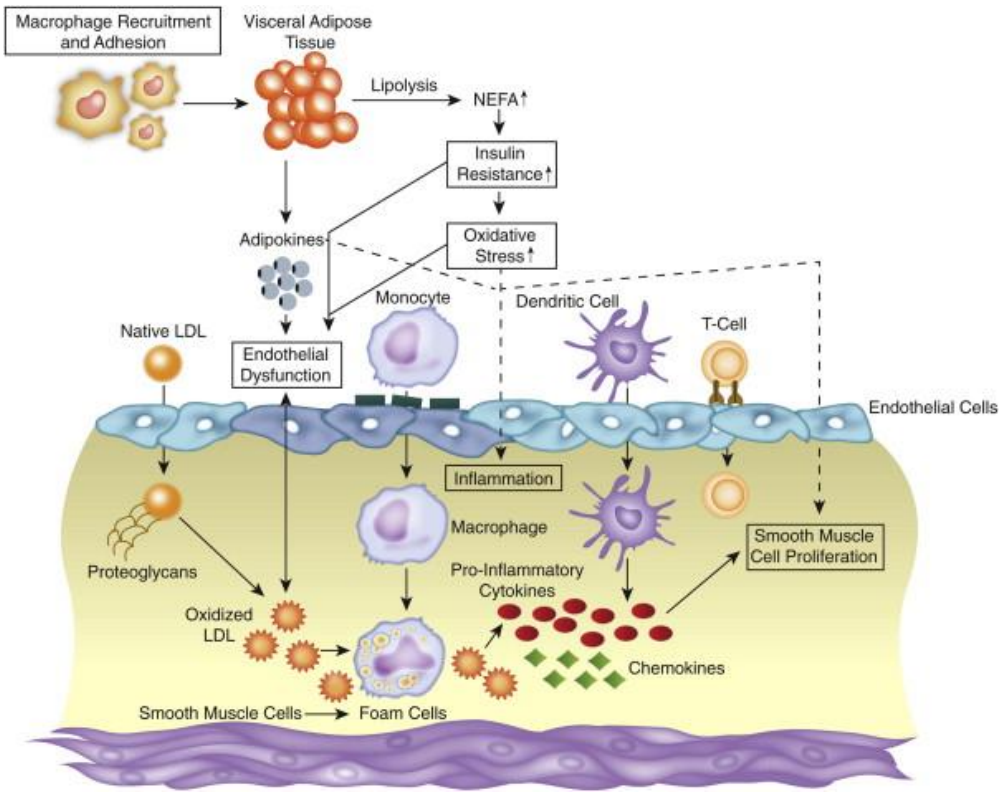

Figura 2. Mecanismos associados à aterosclerose e obesidade. No quadro da obesidade, a dislipidemia e expansão do tecido adiposo visceral, aumentam os níveis de LDL oxidado, que são retidos por macrófagos iniciando resposta inflamatória, proliferação de células musculares lisas e consequentemente progressão da placa aterosclerótica. Além disto, contribuem para o dano endotelial, o aumento na produção de adipocinas pelo tecido adiposo expandido, e o processo de lipólise aumentado que leva à resistência à insulina e estresse oxidativo. Todos estes processos, culminam no recrutamento de outras células imunes no endotélio, levando ao estabelecimento de um estado pró-inflamatório que exacerba a progressão da aterosclerose. Fonte: Hajjar and Gotto, 2013 (56).

1.4 Sistema Renina-Angiotensina

O sistema renina-angiotensina (SRA) é uma cascata enzimática, que exerce função sobre o controle da pressão arterial sistêmica, a homeostase hidroeletrolítica e o remodelamento cardíaco. Além disso, são descritas ações regulatórias sobre processos

metabólicos e inflamatórios, como obesidade, doenças cardiovasculares, *diabetes mellitus* e esteatose hepática gordurosa não alcoólica. Os componentes do SRA são expressos em diferentes graus nos diversos tipos de tecido adiposo (57).

O angiotensinogênio (AGT) é clivado pela renina em Angiotensina I (AngI). Este peptídeo sofre ação da Enzima Conversora de Angiotensina (ECA) e é quebrado, tornando-se Angiotensina II (AngII). A AngII é o principal peptídeo efetor deste sistema e atua por meio de dois receptores principais: Receptor de Angiotensina tipo 1 (AT1) e Receptor de Angiotensina tipo 2 (AT2) (58). Os efeitos clássicos exercidos pela AngII em seu receptor AT1 são: vasoconstrição, fibrose, proliferação celular e inflamação (59). Sabe-se hoje que o SRA tem outros peptídeos ativos que atuam por meio de vias alternativas, principalmente a Angiotensina-(1-7) (Ang-(1-7)), originada pela clivagem da Angiotensina-(1-9) e/ou AngII, pela Enzima Conversora de Angiotensina 2 (ECA2). A Ang-(1-7) atua através do receptor Mas (MasR), constituindo um eixo contra regulatório do SRA (Figura 3).

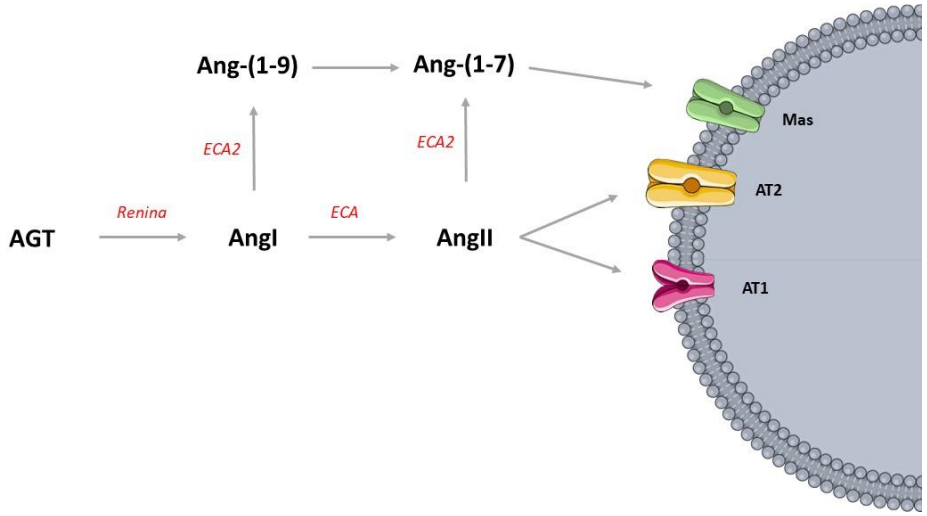

Figura 3. Sistema renina-angiotensina. O angiotensinogênio (AGT) é clivado em Angiotensina I (AngI) pela enzima renina. A AngI, por sua vez, pode ser clivada pela enzima conversora de angiotensina (ECA) em Angiotensina II, ou pela enzima conversora de angiotensina 2 (ECA2) em angiotensina-(1-9) (Ang-(1-9)). A AngII atua por meio de dois principais receptors: Receptor de angiotensina tipo 1 (AT1) e receptor de angiotensina tipo 2 (AT2). A AngII pode ser clivada em Angiotensina-(1-7) (Ang-(1-7) pela ECA2. A Ang-(1-7), por sua vez, atua principalmente através do receptor Mas (Mas). Fonte: arquivo próprio.

No tecido adiposo, o SRA é conhecido por regular crescimento e diferenciação de adipócitos, inflamação, *stress* oxidativo, fluxo sanguíneo local e lipólise/lipogênese. Esses efeitos, somados à influência do próprio tecido adiposo nos vasos sanguíneos, vêm sendo descritos por contribuir com o processo aterosclerótico,, com a disfunção vascular e, por

consequência, com o desencadeamento de processos etiopatogênicos de várias doenças cardiovasculares (60).

1.5 *Cross-talk* entre tecido adiposo pericárdico/perivascular e SRA

Estudos realizados em modelos animais já reportaram a expressão de marcadores do SRA no tecido adiposo perivascular. Galvez-Prieto e colaboradores, em um estudo com ratos da linhagem Wistar, demonstraram a expressão de todos os marcadores do SRA. De maneira interessante, estes mesmos autores demonstraram que o tecido adiposo perivascular de diferentes localizações apresentam padrões de expressão distintos, demonstrando, assim, a especificidade de expressão (61). Acredita-se que o tecido perivascular possa interferir diretamente em funções como metabolismo de lipídeos e pressão sanguínea através da formação local de peptídeos ativos, como a AngII (62). Alguns trabalhos já demonstraram que o tecido perivascular contribui para estados de inflamação, resistência à insulina e consequente disfunção endotelial, especialmente devido à produção aumentada de AngII (63-67).

Os componentes do SRA, ao serem secretados pelo tecido adiposo, atuam de maneira autócrina e/ou parácrina, influenciando o recrutamento de macrófagos, crescimento e proliferação de macrófagos, regulação de adipocinas e controle do tônus vascular (68). Uma vez que a maioria dos vasos sanguíneos é cercada por tecido adiposo, a conexão entre a doença arterial coronariana e a obesidade merece destaque, especialmente devido ao aumento do tecido perivascular no quadro de obesidade (69).

Investigações acerca do papel e das características do tecido adiposo pericárdico no contexto da obesidade e doença arterial coronariana são ausentes na literatura. Além disto, a literatura necessita de estudos realizados com humanos que busquem investigar as relações entre a obesidade, tecidos adiposos pericárdico e perivascular, o sistema renina-angiotensina e doença arterial coronariana.

2 OBJETIVOS

2.1 Objetivo geral

Avaliar as características antropométricas e clínicas de indivíduos eutróficos, com sobrepeso e obesidade e portadores de doença arterial coronariana, bem como a histologia e a expressão de componentes do sistema renina-angiotensina em amostras de tecido adiposo pericárdico e perivascular destes indivíduos.

2.2 Objetivos específicos/questões norteadoras

2.2.1 Pacientes com doença arterial coronariana eutróficos e com sobrepeso ou obesidade apresentam diferenças quanto ao perfil clínico e bioquímico?

2.2.2 Pacientes com doença arterial coronariana eutróficos e com sobrepeso ou obesidade diferem no perfil histológico dos tecidos adiposos pericárdico e perivascular?

2.2.3 Pacientes com doença arterial coronariana eutróficos e com sobrepeso ou obesidade diferem na expressão de marcadores chave do sistema renina-angiotensina nos tecidos adiposos pericárdico e perivascular?

3 PRODUTO

3.1 Produto 1: *Clinical And Adipose Tissue Profile Of Eutrophic And Obese Patients With Advanced Coronary Disease,* formatado segundo as normas para publicação do periódico Life Sciences.

3.1 PRODUTO 1

Clinical And Adipose Tissue Profile Of Eutrophic And Obese Patients With Advanced Coronary Disease

Deborah de Farias Lelis[1], Thaisa Soares Crespo[1], Victor Hugo Dantas Guimarães[1], Antônio Sérgio Barcala Jorge[2], João Marcus Oliveira Andrade[1], Flavio Donizete Gonçalves[2], Alfredo Maurício Batista de Paula[1], André Luiz Sena Guimarães[1], Lucyana Conceição Farias[1], Sergio Henrique Sousa Santos[1,3*]

[1] Laboratory of Health Science, Postgraduate Program in Health Sciences, Universidade Estadual de Montes Claros, Montes Claros, Minas Gerais, Brazil.

[2] Dilson Godinho Hospital, Surgery Department, Montes Claros, Minas Gerais, Brazil.

[3] Institute of Agriculture Sciences. Departments of Food Engineering; Universidade Federal de Minas Gerais, Minas Gerais, Brazil.

Correspondence to: Sérgio H S Santos. Laboratory of Health Science. Universidade Estadual de Montes Claros. Av. Cula Mangabeira 562. 31401-001, Montes Claros, MG, Brazil. FAX/Phone: (55-38) 3229-8327. E-mail: sergiosousas@hotmail.com

Abstract

Aims: The present study aimed to evaluate the anthropometric and clinical characteristics of eutrophic and individuals with overweight or obesity with coronary artery disease (CAD), as well as the histology and expression of the renin-angiotensin system (RAS) components in the pericardial and perivascular adipose tissue samples from these individuals. Main methods: 19 samples of pericardial and perivascular adipose tissue, blood and associated clinical data, were obtained from patients submitted to coronary artery bypass grafting surgery. The samples were divided into eutrophic and overweight/obesity, according to the Body Mass Index. The following parameters were evaluated: Clinical data, biochemical parameters, adipocyte area of the pericardial and perivascular adipose tissues after H&E staining, and the expression of RAS components by real - time quantitative PCR. Key findings: The main results showed that in our research conditions, the adipocytes area in the perivascular adipose tissue and CRP ultra-sensitive serum levels were increased in overweight/obese individuals as compared to eutrophic individuals, pointing to obesity-associated inflammatory and hypertrophic states. The clinical and biochemical profile was no different between groups, as well as the RAS markers evaluated (Angiotensin II Type 1 receptor and Angiotensin Converting Enzyme 2) that remained similarly expressed between tissues/groups. Significance: In summary, overweight/obese individuals seems to have larger adipocytes in the perivascular adipose tissue and increased CRP ultra-sensitive serum levels, contributing factors to the atherosclerotic process. However, larger studies should be perform to confirm our findings and provide additional investigations.

Keywords: Obesity. White adipose tissue. Cardiovascular disease. Renin-angiotensin system.

Introduction

Obesity, characterized by abnormal fat accumulation, commonly due to the imbalance between energy consumption and expenditure, is considered a pandemic worldwide (Swinburn et al. , 2011). According to the World Health Organization, in 2016, more than 2 billion adults were overweight or obese (World Health Organization, 2018). Obesity is a risk factor for several conditions such as depression, type 2 diabetes, cardiovascular disease, certain types of cancers and mortality (Hruby and Hu, 2015).

The white adipose tissue, main body fat reservoir, is the protagonist organ in obesity, and considered an important endocrine organ. Through the production, secretion and expression of several bioactive molecules, the white adipose tissue regulates metabolism, energy intake, and fat storage (Greenberg and Obin, 2006). In obesity, however, the adipocytes undergo process such as hyperplasia and hypertrophy, which have a detrimental effect on metabolism leading to conditions such as insulin resistance and inflammation (Greenberg and Obin, 2006). Moreover, the adipose tissue have several different locations through the body and its depots are different in many characteristics (Bjorndal et al. , 2011). The perivascular and pericardial adipose tissues deserve especial attention due to their close proximity with important organs of the cardiovascular system, the arteries and heart, respectively.

Cardiovascular diseases comprise an important group of obesity-associated disorders, and more specifically, the coronary artery disease (CAD), is the leading global cause of mortality, deserving attention (Khera and Kathiresan, 2017). Coronary artery disease is considered a chronic inflammatory disease that can lead to coronary arteries impairment by hindering the oxygen supply to the heart (Sayols-Baixeras et al. , 2014). CAD starts with atherosclerosis, which is the accumulation of lipids, inflammatory/immune cells and fibrous molecules in the arteries walls (Sayols-Baixeras et al., 2014).

The renin-angiotensin system (RAS), an enzymatic cascade that regulates the hydroelectrolytic state and hemodynamics systems in the body, might be a possible link connecting obesity, adipose tissue and coronary artery disease by its influence on the fibrinolytic balance, inflammatory status, endothelial function and plaque stability (Hammoud et al. , 2007). During obesity, the classic components of this system are usually overexpressed and exert deleterious effects in different physiological functions (Kalupahana and Moustaid-Moussa, 2012).

In this perspective, we aimed to evaluated differences in the perivascular and pericardial adipose tissues, by investigating their adipocytes size and renin-angiotensin system markers expression between eutrophic and overweight/obese individuals with CAD.

Methods

Human samples

The biological samples (perivascular adipose tissue (PVAT), surrounding the saphenous vein, pericardial adipose tissue (PCAD), located in the visceral pericardium and on the external surface of the parietal pericardium), and clinical-associated data were obtained from the Bank of Human Biological Materials of North of Minas Gerais (Institutional Biobank-State University of Montes Claros, Brazil/National Commission of Ethics in Research, Registration: B-013). Ethical approval for this study was obtained from the relevant Institutional Review Board (Protocol No. 66566117.8.0000.5146). Clinical and anthropometric variables were collected in a clinical questionnaire.

The samples were obtained from individuals assisted in public health centers, with coronary artery disease, submitted to elective coronary artery bypass grafting (CABG), that accepted to donate the samples to the biological biobank aforementioned (n=19). The samples were divided into eutrophic (Body Mass Index $\leq$ 24.9 Kg/m^2) and overweight/obese (Body Mass Index > 24.9 Kg/m^2) (Nuttall, 2015), according to the samples-associated clinical data. Anthropometric measures, including Bod y Weight, Body-Mass Index (BMI), Waist Circumference (WC), Neck Circumference (NC), Hip Circumference (HC) and Waist-Hip Ratio (WHR) were evaluated.

Biochemical analysis

The blood samples used were obtained by venipuncture (after a 12-hour fasting period, before anesthesia) and biochemical parameters were assessed. Serum levels of total cholesterol (mg/mL), high-density lipoprotein cholesterol (HDL-c) (mg/mL), triglycerides (mg/mL), fasting glucose (mg/mL), and ultra-sensitive C-reactive protein (mg/mL) were assayed using enzymatic kits (Wiener®, Argentina) on a Wiener BT-3000 plus Chemistry Analyzer (Wiener®, Argentina) as previously reported (de Almeida Pinheiro et al. , 2017).

Low-density lipoprotein (LDL-c) concentrations were also calculated by the Friedewald's formula (Friedewald et al. , 1972).

Histological analysis

The perivascular and pericardial adipose tissue fragments were fixed in paraformaldehyde 4% solution and paraffin embedded. For the hematoxylin & eosin staining the tissues were dewaxed, hydrated and stained. The slides were evaluated under a conventional microscope (Axioskop 40). Images from the adipose tissues were taken with x10 ocular and x40 objective lenses on a ZEISS AxioCam MRc Digital Camera. We calculated the adipocytes area from five representative fields from each slide.

Real time quantitative polymerase chain reaction (qRT-PCR)

The total RNA was extracted from the perivascular and pericardial adipose tissue samples using Trizol reagent (Invitrogen Corp.®, San Diego, California, USA). Following, the total RNA was treated with DNAse and reverse transcribed with M-MLV (Invitrogen Corp.®) using random hexamer primers and oligodT. The mRNA expression was evaluated by quantitative real time PCR using SYBR Green reagent (Applied Biosystems®, USA) in a PlusOne platform (Applied Biosystems®) with specific primer sequences. The endogenous Beta actin (β-Act) (internal control): Forward 5'- AGGCACCAGGGCGTGAT-3' and Reverse: 5'-GCCCACATAGGAATCCTTCTGAC-3' (Chakrabarti et al. , 2015), Angiotensin II type 1 receptor (AT1): Forward: 5' ATA CAC CTG GTG CCG ACT TTC TG 3' and Reverse: 5' GGG CGC GGG TTT GAT ATT TGA CA 3'; Angiotensin-converting enzyme 2 (ACE2): Forward: 5' CAT TGG AGC AAG TGT TGG ATC TT 3' and Reverse: 5' GAG CTA ATG CAT GCC ATT CTC A 3' (Konoshita et al. , 2006). Samples were analyzed 2-delta-delta Ct method (Livak and Schmittgen, 2001).

Statistical analysis

The statistical analysis were performed at Graph Pad Prism software (version 5.0) and Statistical Package for the Social Sciences - SPSS software (version 18.0). The clinical data was analyzed as frequencies (mean ± standard error for continuous variables and percentages

for categorical variables) and the chi-square test was applied to verify differences among them. The statistical significance of differences was assessed by one-way ANOVA followed by Bonferroni post-test for the anthropometric, adipocytes area and biochemical data. Two-way ANOVA followed by Bonferroni post-test was applied to evaluate the statistical difference for the mRNA expression analysis. The statistical significance was set as $p < 0.05$.

Results

The patients' clinical-associated characteristics are described in Table 1. According to the clinical data, we divided the samples into eutrophic (BMI≤24.9) and overweight/obese (BMI>24.9) for the further analyzes. The eutrophic and overweight/obese groups presented no statistically significant differences regarding mean age, gender, *diabetes mellitus*, hypertension, dyslipidemia, metabolic syndrome (according to the National Cholesterol Education Program (NCEP) (Table 1). The overweight/obese individuals on the other hand, presented statistically significant increased body weight, body mass index, neck, waist and hip circumference and also increased waist-hip ratio (Figure 1A-F).

Table 1. Clinical characteristics between eutrophic and overweight/obese individuals with CAD.

Variables	**All (n=19)**	**Eutrophic (n=10)**	**Overweight/obese (n=9)**	**p-value**
Age (Mean ± SD)	64.52 ± 8.84	68.7 ± 6.53	59.88 ± 9.04	0.792
Gender (n (%))				0.091
Female	6 (31.6)	5 (50)	1 (11)	
Male	13 (68.4)	5 (50)	8 (89)	
Diabetes Mellitus (n (%))				0.667
Yes	10 (52.6)	2 (20)	2 (22)	
No	9 (47.4)	8 (80)	7 (78)	
Dyslipidemia (n (%))*				0.594
Yes	4 (21.1)	6 (60)	4 (44)	
No	15 (78.9)	2 (20)	2 (22)	
Hypertension (n (%))				0.605
Yes	10 (52.6)	6 (60)	5 (55)	
No	4 (21.1)	4 (40)	4 (44)	
Metabolic Syndrome (n (%))*				0.657
Yes	11 (57.9)	5 (50)	4 (44)	
No	8 (42.1)	3 (33)	2 (22)	

* missing data.

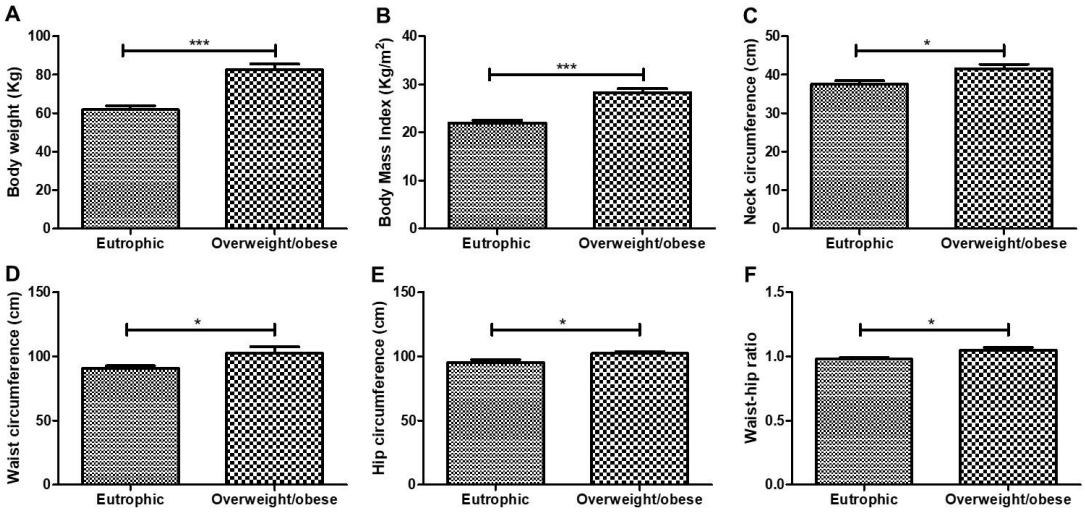

Figure 1. Individuals anthropometric profile. A) Body weight (Kg); B) Body Mass Index (Kg/m^2); C) Neck circumference (cm); D) Waist circumference (cm); E) Hip circumference (cm); Waist-hip ratio. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ versus indicated groups by the bars (One-way ANOVA).

Regarding the biochemical profile assessed in the blood samples, we observed similar levels of total cholesterol, triglycerides, high-density lipoprotein, low-density lipoprotein and glucose between eutrophic and overweight/obese individuals (Figure 2A-E). In contrast, the quantitative ultra-sensitive C-reactive protein levels were increased in overweight/obese individuals as compared to eutrophic (Figure 2F).

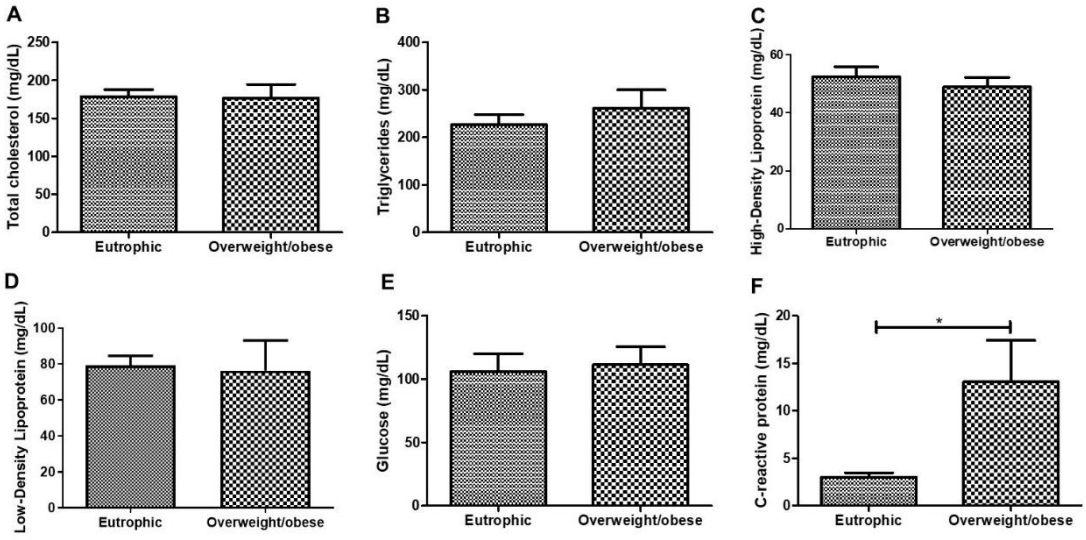

Figure 2. Eutrophic and overweight/obese Individuals biochemical profile. A) Serum total cholesterol (mg/dL); B) Serum triglycerides (mg/dL); C) Serum high-density lipoprotein

(HDL-c) (mg/dL); D) Serum low-density lipoprotein (LDL-c) (mg/dL); E) Fasting serum glucose (mg/dL); F) Serum ultra-sensitive C-reactive protein (mg/dL). * $p < 0.05$ versus indicated groups by the bars (One-way ANOVA).

The pericardial and perivascular adipose tissue histological analysis evidenced similar adipocyte area between eutrophic and overweight/obese individuals for the pericardial adipose tissue (Figure 3A-C). The perivascular adipose tissue adipocyte area in contrast, was found statistically increased in the overweight/obese individuals as compared to eutrophic (Figure 3D-E).

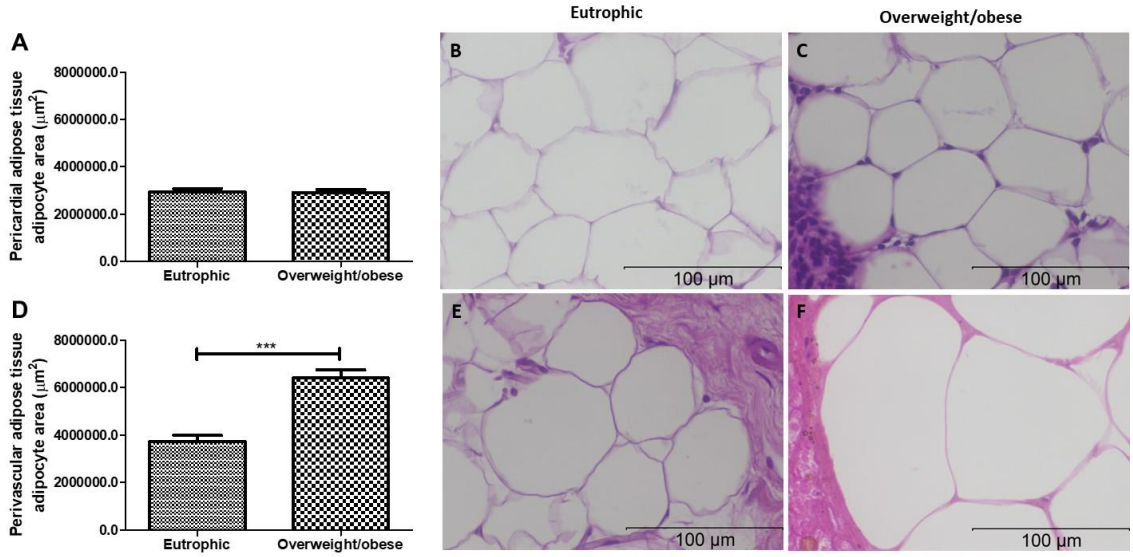

Figure 3. Pericardial and perivascular adipose tissues adipocyte area. A) Pericardial adipose tissue adipocyte area (μm^2) from eutrophic and overweight/obese individuals; B) Pericardial adipose tissue H&E staining from eutrophic individuals; C) Pericardial adipose tissue H&E staining from overweight/obese individuals; D) Perivascular adipose tissue adipocyte area (μm^2); E) Perivascular adipose tissue H&E staining from eutrophic individuals; F) Perivascular adipose tissue H&E staining from overweight/obese individuals. Scale bar: 100 µm, 40x. *** $p < 0.001$ versus the group indicated by the bars (One-way ANOVA).

The renin-angiotensin system components expression profile between the pericardial and perivascular adipose tissues and between eutrophic and overweight/obese individuals presented no statistically significant differences (Figure 4A and B).

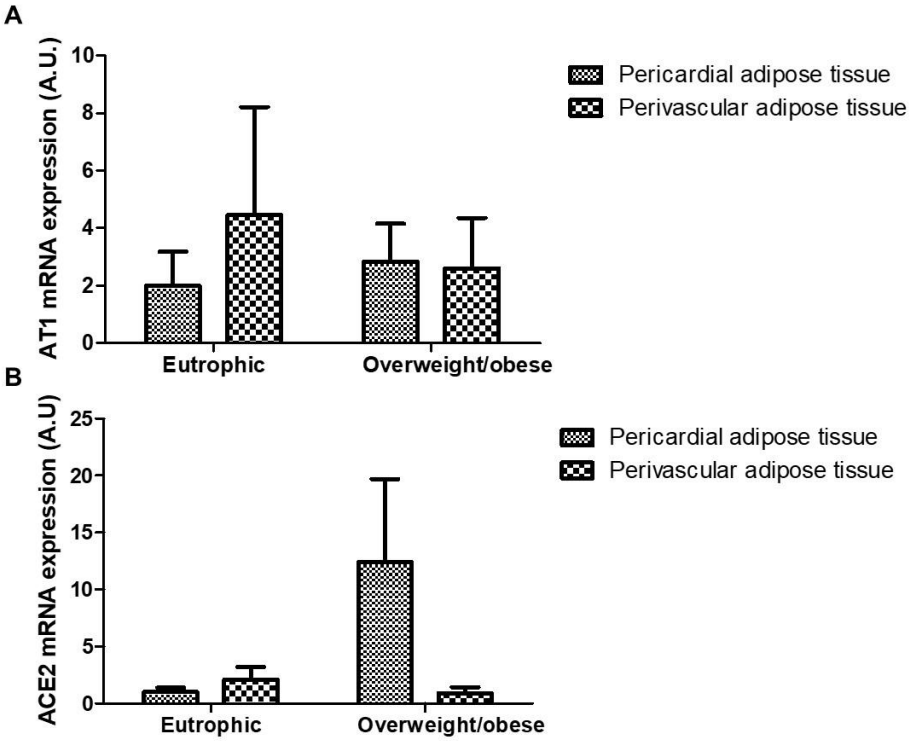

Figure 4. Renin-angiotensin system markers mRNA expression in the pericardial and perivascular adipose tissue from eutrophic and overweight/obese individuals. A) Angiotensin type 1 receptor (AT1) mRNA expression (A.U.); B) Angiotensin-converting enzyme 2 (ACE2) mRNA expression (A.U.).

Discussion

The main findings of the present study revealed that overweight/obese individuals have increased adipocytes in the perivascular adipose tissue as compared to eutrophic individuals, while the adipocytes area in the pericardial adipose tissue remained similar between groups. The renin-angiotensin system components (AT1 and ACE2) expression was similar among groups and different tissue samples (perivascular *vs*. pericardial).

The first noteworthy finding in our study is the individuals age. Although the chi-square test did not revealed statistical significant differences between overweight/obese and eutrophic individuals we could observe a 10-year difference in the mean age. Among the patients with coronary artery disease eutrophic individuals tend to be older than overweight/obese at the time of intervention. This observation, yet controversial, has been extensively discussed in the literature for both acute and stable coronary artery disease patients (Bucholz et al. , 2012, Camprubi et al. , 2012, Kang et al. , 2010, Kosuge et al. , 2008). Interestingly, a few studies report worse outcomes for eutrophic individuals as compared to overweight/obese, which may be related to their age difference (Kang et al.,

2010, Oreopoulos et al. , 2009, Poston et al. , 2004). Furthermore, the literature discuss other factors that may be linked to the "better" prognosis observed among overweight/obese individuals, such as greater adherence to treatment guidelines (Akin et al. , 2012, Gurm et al. , 2002), the vessels diameter (which are claimed to be higher in overweight/obese individuals) (Foley et al. , 1994, Schunkert et al. , 1999), the antithrombotic weight-adjusted dosage (overweight/obese individuals tend to have less bleeding effects than normal weight individuals) (Powell et al. , 2003), among others.

On the other hand, obesity is widely known to be one of the most important risk factor for cardiovascular diseases, as it is associated with several hemodynamic and metabolic abnormalities (Alpert et al. , 2016). The scientific literature emphasize the measurement of adiposity in order to characterize the obesity state, as central and general obesity are important predictors of cardiovascular diseases (Alpert et al., 2016, Jensen et al. , 2014, Lee et al. , 2008). In our study we observed increased body weight, BMI, WC, NC, HC and WHR in overweight/obese individuals as compared to eutrophic. These corroborated previous reports that showed a positive correlation between the measurements of central obesity and cardiovascular risk (Berg and Scherer, 2005, Goh et al. , 2014).

Obesity is independently associated with cardiovascular diseases and the main possible associated mechanisms are: obesity-mediated free fatty acid turnover, hypercoagulable and inflammatory states (Festa et al. , 2001, McMahan et al. , 2007), and augmented insulin resistance (Artham et al. , 2009). Interestingly, although the lipid profile was similar between eutrophic and overweight/obese individuals, we observed increased ultra-sensitive C-reactive protein levels in overweight/obese individuals. Coronary artery disease is an inflammatory condition characterized by vascular endothelium damage, atherosclerotic plaque formation and disruption (Madjid and Willerson, 2011), which may lead to more severe consequences such as myocardial infarction. Among the inflammatory markers, CRP is the most studied in cardiovascular diseases (Arroyo-Espliguero et al. , 2009, Koenig et al. , 1999, Raposeiras Roubin et al. , 2013, Ridker et al. , 1997) due to its ability to reduce nitric oxide synthase and prostacyclin synthase expression, enhance the LDL-c uptake by macrophages and increase the expression of adhesion molecules on endothelial cells (Mehta et al. , 2007). Additionally, CRP facilitates monocytes infiltration, inhibits fibrinolysis (de Maat and Trion, 2004). Increased CRP levels are believed to indicate a poor cardiovascular prognosis (Casas et al. , 2008, Ridker, 2005). As our investigation does not include the participants follow-up we are not be able to infer the CRP association with

additional cardiovascular outcomes, although other studies already described its value in predicting the cardiovascular prognosis after an event (Auer et al. , 2002, Habib et al. , 2011).

Interestingly, CRP levels are not only strongly associated with coronary artery disease but also with obesity. Aronson and cols. on a population-based cross-sectional study with 1,929 individuals concluded that obesity is the major factor associated with increased CRP levels in patients with metabolic syndrome (Aronson et al. , 2004), corroborating similar findings previously reported (Visser et al. , 1999).

As we have been discussing, obesity is an important risk factor for cardiovascular diseases and the white adipose tissue deserves attention in this scenario as it is considered the protagonist organ due to fat accumulation (Coelho et al. , 2013). The adipose tissue is an endocrine organ that exerts local and systemic effects, especially inducing a low-grade inflammatory state in overweight/obesity conditions (Coelho et al., 2013).

First, we have evaluated the adipocytes area from both adipose tissue depots (perivascular and pericardial). We observed increased adipocytes in the perivascular adipose tissue from overweight/obese individuals as compared to eutrophic individuals. As the individuals gain weight, the adipocytes tend to enlarge (hypertrophy), and store larger amounts of fat, however, adipocytes hypertrophy is associated with cellular stress, decreased metabolic flexibility (Muir et al. , 2016), increased production of adipokines, free fatty acids, and inflammatory mediators (de Ferranti and Mozaffarian, 2008). It has been reported in the literature that adipocyte size is a significant predictor of macrophage accumulation (Weisberg et al. , 2003), and activates different immune cells (Verboven et al. , 2018), which in turn may augment the inflammatory state commonly observed in obesity and cardiovascular disease. The importance of this finding is that as the adipose tissue may freely communicate with the vessels inner layers (Omar et al. , 2014), and in adverse conditions such as obesity/CAD, it may augment the endothelial damage, thus favoring the atherosclerotic plaque formation/disruption. The adipose tissue may communicate with the vessels via the *vasa vasorum* system, which especially in the human PVAT from the saphenous vein (the one used in the present investigation), is extensive and more prolific (Loesch and Dashwood, 2018). In a mouse-model study, it was showed that the PVAT may have a direct influence on vessel contractility via dependent and/or independent-endothelium mechanisms (Loesch and Dashwood, 2018). The angiotensin II (AngII) is pointed as a potential important mechanism by which the PVAT may influence vasocontractility, thus emphasizing the importance of this metabolic organ on cardiovascular disease.

The renin-angiotensin system is an important regulator of whole-body hemodynamics, metabolism and volume status (Hammoud et al., 2007). However, this system is also involved in the pathogenesis of cardiovascular diseases, especially coronary artery disease, by its influence on fibrinolytic balance, inflammatory status, atherosclerotic plaque stability, and vascular endothelial function (Tsikouris and Cox, 2003). Interestingly, angiotensin-converting enzyme inhibitors (ACEi) and angiotensin receptor blockers (ARBs) have been shown to exert beneficial effects in the management of cardiovascular complications associated with CAD (2004, Fox and Investigators, 2003, Heart Outcomes Prevention Evaluation Study et al. , 2000), although yet controversial. Due to the RAS importance on CAD, we further evaluated the perivascular and pericardial adipose tissue and assessed the renin-angiotensin system key components expression profile. We observed no differences between eutrophic and overweight/obese individuals and no differences between tissues (perivascular vs. pericardial adipose tissue). Based on these results we might say that locally, obesity or the adipose tissue specific depot do not influence the RAS expression and should not consist in a target for therapeutic approaches. However, our study has some limitations, such as the small sample size, raising the need for further analyzes on the RAS expression profile in larger sample size.

In summary, we showed that the clinical and biochemical profile of CAD patients is no different between eutrophic and overweight/obese individuals, except for the CRP levels, which are increased in overweight/obese, thus indicating the obesity-associated inflammatory status. The adipose tissue analysis revealed that overweight/obese individuals have increased perivascular adipocytes as compared to eutrophic, thus indicating a weight-related dysfunction in this tissue. The renin-angiotensin system components expression was similar between tissues (perivascular and pericardial adipose tissue) and BMI stratifications (eutrophic vs. overweight/obese). The local and systemic importance of the white adipose tissue may constitute a promising approach in the prevention or treatment of cardiovascular diseases, especially coronary artery disease, thus further studies and analysis are needed to elucidate this organ role during disease.

Funding

This work was supported by the Coordenadoria de Aperfeiçoamento do Pessoal de Nível Superior (CAPES) and Conselho Nacional de Desenvolvimento Científico (CNPQ) and Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG).

Conflicts of interest

The authors have nothing to disclose.

References

Angiotensin-Converting–Enzyme Inhibition in Stable Coronary Artery Disease. New England Journal of Medicine. 2004;351:2058-68.

Akin I, Tolg R, Hochadel M, Bergmann MW, Khattab AA, Schneider S, et al. No evidence of "obesity paradox" after treatment with drug-eluting stents in a routine clinical practice: results from the prospective multicenter German DES.DE (German Drug-Eluting Stent) Registry. JACC Cardiovascular interventions. 2012;5:162-9.

Alpert MA, Omran J, Bostick BP. Effects of Obesity on Cardiovascular Hemodynamics, Cardiac Morphology, and Ventricular Function. Current obesity reports. 2016;5:424-34.

Aronson D, Bartha P, Zinder O, Kerner A, Markiewicz W, Avizohar O, et al. Obesity is the major determinant of elevated C-reactive protein in subjects with the metabolic syndrome. International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity. 2004;28:674-9.

Arroyo-Espliguero R, Avanzas P, Quiles J, Kaski JC. Predictive value of coronary artery stenoses and C-reactive protein levels in patients with stable coronary artery disease. Atherosclerosis. 2009;204:239-43.

Artham SM, Lavie CJ, Milani RV, Ventura HO. Obesity and hypertension, heart failure, and coronary heart disease-risk factor, paradox, and recommendations for weight loss. The Ochsner journal. 2009;9:124-32.

Auer J, Berent R, Lassnig E, Eber B. C-reactive protein and coronary artery disease. Japanese heart journal. 2002;43:607-19.

Berg AH, Scherer PE. Adipose tissue, inflammation, and cardiovascular disease. Circulation research. 2005;96:939-49.

Bjorndal B, Burri L, Staalesen V, Skorve J, Berge RK. Different adipose depots: their role in the development of metabolic syndrome and mitochondrial response to hypolipidemic agents. Journal of obesity. 2011;2011:490650.

Bucholz EM, Rathore SS, Reid KJ, Jones PG, Chan PS, Rich MW, et al. Body mass index and mortality in acute myocardial infarction patients. The American journal of medicine. 2012;125:796-803.

Camprubi M, Cabrera S, Sans J, Vidal G, Salvado T, Bardaji A. Body mass index and hospital mortality in patients with acute coronary syndrome receiving care in a university hospital. Journal of obesity. 2012;2012:287939.

Casas JP, Shah T, Hingorani AD, Danesh J, Pepys MB. C-reactive protein and coronary heart disease: a critical review. Journal of internal medicine. 2008;264:295-314.

Chakrabarti R, Sanyal S, Ghosh A, Bhar K, Das C, Siddhanta A. Phosphatidylinositol-4-phosphate 5-Kinase 1alpha Modulates Ribosomal RNA Gene Silencing through Its Interaction with Histone H3 Lysine 9 Trimethylation and Heterochromatin Protein HP1-alpha. The Journal of biological chemistry. 2015;290:20893-903.

Coelho M, Oliveira T, Fernandes R. Biochemistry of adipose tissue: an endocrine organ. Archives of medical science : AMS. 2013;9:191-200.

de Almeida Pinheiro T, de Almeida Pinheiro T, Feltenberger JD, Andrade JMO, Neves Ferreira EC, De Farias Lelis D, et al. Effects of Resveratrol and ACE Inhibitor Enalapril on Glucose and Lipid Profiles in Mice. Protein and peptide letters. 2017;24:854-60.

de Ferranti S, Mozaffarian D. The perfect storm: obesity, adipocyte dysfunction, and metabolic consequences. Clinical chemistry. 2008;54:945-55.

de Maat MP, Trion A. C-reactive protein as a risk factor versus risk marker. Current opinion in lipidology. 2004;15:651-7.

Festa A, D'Agostino R, Jr., Williams K, Karter AJ, Mayer-Davis EJ, Tracy RP, et al. The relation of body fat mass and distribution to markers of chronic inflammation. International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity. 2001;25:1407-15.

Foley DP, Melkert R, Serruys PW. Influence of coronary vessel size on renarrowing process and late angiographic outcome after successful balloon angioplasty. Circulation. 1994;90:1239-51.

Fox KM, Investigators EUtOrocewPiscAd. Efficacy of perindopril in reduction of cardiovascular events among patients with stable coronary artery disease: randomised, double-blind, placebo-controlled, multicentre trial (the EUROPA study). Lancet. 2003;362:782-8.

Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clinical chemistry. 1972;18:499-502.

Goh LGH, Dhaliwal SS, Welborn TA, Lee AH, Della PR. Anthropometric measurements of general and central obesity and the prediction of cardiovascular disease risk in women: a cross-sectional study. BMJ Open. 2014;4:e004138.

Greenberg AS, Obin MS. Obesity and the role of adipose tissue in inflammation and metabolism. The American journal of clinical nutrition. 2006;83:461S-5S.

Gurm HS, Whitlow PL, Kip KE, Investigators B. The impact of body mass index on short- and long-term outcomes inpatients undergoing coronary revascularization. Insights from the bypass angioplasty revascularization investigation (BARI). Journal of the American College of Cardiology. 2002;39:834-40.

Habib SS, Kurdi MI, Al Aseri Z, Suriya MO. CRP levels are higher in patients with ST elevation than non-ST elevation acute coronary syndrome. Arquivos brasileiros de cardiologia. 2011;96:13-7.

Hammoud RA, Vaccari CS, Nagamia SH, Khan BV. Regulation of the renin-angiotensin system in coronary atherosclerosis: a review of the literature. Vascular health and risk management. 2007;3:937-45.

Heart Outcomes Prevention Evaluation Study I, Yusuf S, Sleight P, Pogue J, Bosch J, Davies R, et al. Effects of an angiotensin-converting-enzyme inhibitor, ramipril, on cardiovascular events in high-risk patients. The New England journal of medicine. 2000;342:145-53.

Hruby A, Hu FB. The Epidemiology of Obesity: A Big Picture. PharmacoEconomics. 2015;33:673-89.

Jensen MD, Ryan DH, Apovian CM, Ard JD, Comuzzie AG, Donato KA, et al. 2013 AHA/ACC/TOS guideline for the management of overweight and obesity in adults: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines and The Obesity Society. Circulation. 2014;129:S102-38.

Kalupahana NS, Moustaid-Moussa N. The renin-angiotensin system: a link between obesity, inflammation and insulin resistance. Obesity reviews : an official journal of the International Association for the Study of Obesity. 2012;13:136-49.

Kang WY, Jeong MH, Ahn YK, Kim JH, Chae SC, Kim YJ, et al. Obesity paradox in Korean patients undergoing primary percutaneous coronary intervention in ST-segment elevation myocardial infarction. Journal of cardiology. 2010;55:84-91.

Khera AV, Kathiresan S. Genetics of coronary artery disease: discovery, biology and clinical translation. Nature Reviews Genetics. 2017;18:331.

Koenig W, Sund M, Frohlich M, Fischer HG, Lowel H, Doring A, et al. C-Reactive protein, a sensitive marker of inflammation, predicts future risk of coronary heart disease in initially healthy middle-aged men: results from the MONICA (Monitoring Trends and Determinants in Cardiovascular Disease) Augsburg Cohort Study, 1984 to 1992. Circulation. 1999;99:237-42.

Konoshita T, Wakahara S, Mizuno S, Motomura M, Aoyama C, Makino Y, et al. Tissue gene expression of renin-angiotensin system in human type 2 diabetic nephropathy. Diabetes care. 2006;29:848-52.

Kosuge M, Kimura K, Kojima S, Sakamoto T, Ishihara M, Asada Y, et al. Impact of body mass index on in-hospital outcomes after percutaneous coronary intervention for ST segment elevation acute myocardial infarction. Circulation journal : official journal of the Japanese Circulation Society. 2008;72:521-5.

Lee CM, Huxley RR, Wildman RP, Woodward M. Indices of abdominal obesity are better discriminators of cardiovascular risk factors than BMI: a meta-analysis. Journal of clinical epidemiology. 2008;61:646-53.

Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods. 2001;25:402-8.

Loesch A, Dashwood MR. Nerve-perivascular fat communication as a potential influence on the performance of blood vessels used as coronary artery bypass grafts. Journal of cell communication and signaling. 2018;12:181-91.

Madjid M, Willerson JT. Inflammatory markers in coronary heart disease. British medical bulletin. 2011;100:23-38.

McMahan CA, McGill HC, Gidding SS, Malcom GT, Newman WP, Tracy RE, et al. PDAY risk score predicts advanced coronary artery atherosclerosis in middle-aged persons as well as youth. Atherosclerosis. 2007;190:370-7.

Mehta JL, Sukhija R, Romeo F, Sepulveda JL. Value of CRP in coronary risk determination. Indian heart journal. 2007;59:173-7.

Muir LA, Neeley CK, Meyer KA, Baker NA, Brosius AM, Washabaugh AR, et al. Adipose tissue fibrosis, hypertrophy, and hyperplasia: Correlations with diabetes in human obesity. Obesity. 2016;24:597-605.

Nuttall FQ. Body Mass Index: Obesity, BMI, and Health: A Critical Review. Nutr Today. 2015;50:117-28.

Omar A, Chatterjee TK, Tang Y, Hui DY, Weintraub NL. Proinflammatory phenotype of perivascular adipocytes. Arteriosclerosis, thrombosis, and vascular biology. 2014;34:1631-6.

Oreopoulos A, McAlister FA, Kalantar-Zadeh K, Padwal R, Ezekowitz JA, Sharma AM, et al. The relationship between body mass index, treatment, and mortality in patients with established coronary artery disease: a report from APPROACH. European heart journal. 2009;30:2584-92.

Paffen E, DeMaat MP. C-reactive protein in atherosclerosis: A causal factor? Cardiovascular research. 2006;71:30-9.

Poston WS, Haddock CK, Conard M, Spertus JA. Impact of obesity on disease-specific health status after percutaneous coronary intervention in coronary disease patients. International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity. 2004;28:1011-7.

Powell BD, Lennon RJ, Lerman A, Bell MR, Berger PB, Higano ST, et al. Association of body mass index with outcome after percutaneous coronary intervention. The American journal of cardiology. 2003;91:472-6.

Raposeiras Roubin S, Barreiro Pardal C, Roubin-Camina F, Ocaranza Sanchez R, Alvarez Castro E, Paradela Dobarro B, et al. High-sensitivity C-reactive protein predicts adverse outcomes after non-ST-segment elevation acute coronary syndrome regardless of GRACE risk score, but not after ST-segment elevation myocardial infarction. Revista portuguesa de cardiologia : orgao oficial da Sociedade Portuguesa de Cardiologia = Portuguese journal of cardiology : an official journal of the Portuguese Society of Cardiology. 2013;32:117-22.

Ridker PM. C-reactive protein, inflammation, and cardiovascular disease: clinical update. Texas Heart Institute journal. 2005;32:384-6.

Ridker PM, Cushman M, Stampfer MJ, Tracy RP, Hennekens CH. Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. The New England journal of medicine. 1997;336:973-9.

Sayols-Baixeras S, Lluis-Ganella C, Lucas G, Elosua R. Pathogenesis of coronary artery disease: focus on genetic risk factors and identification of genetic variants. The application of clinical genetics. 2014;7:15-32.

Schunkert H, Harrell L, Palacios IF. Implications of small reference vessel diameter in patients undergoing percutaneous coronary revascularization. Journal of the American College of Cardiology. 1999;34:40-8.

Swinburn BA, Sacks G, Hall KD, McPherson K, Finegood DT, Moodie ML, et al. The global obesity pandemic: shaped by global drivers and local environments. Lancet. 2011;378:804-14.

Tsikouris JP, Cox CD. Pharmacologic blockade of the renin-angiotensin system: vascular benefits beyond commonly understood pharmacologic actions. Pharmacotherapy. 2003;23:1141-52.

Verboven K, Wouters K, Gaens K, Hansen D, Bijnen M, Wetzels S, et al. Abdominal subcutaneous and visceral adipocyte size, lipolysis and inflammation relate to insulin resistance in male obese humans. Scientific Reports. 2018;8:4677.

Visser M, Bouter LM, McQuillan GM, Wener MH, Harris TB. Elevated C-reactive protein levels in overweight and obese adults. Jama. 1999;282:2131-5.

Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW, Jr. Obesity is associated with macrophage accumulation in adipose tissue. The Journal of clinical investigation. 2003;112:1796-808.

World Health Organization. Obesity and overweight. 2018.

4 CONCLUSÕES

Conclui-se os pacientes com sobrepeso/obesidade apresentam níveis aumentados de PCR ultrassensível e área de adipócitos aumentada no tecido adiposo perivascular. Os níveis de expressão de componentes do sistema renina-angiotensina não foram diferentes entre os dois grupos de estudo, mas limitações na casuística podem ser um fator comprometedor na obtenção de outros resultados para essa variável. Os marcadores antropométricos associados à obesidade foram marcadamente aumentados no grupo de indivíduos com sobrepeso/obesidade e parecem ser o mais importante fator de risco isolado para a ocorrência de CAD, sobretudo quando se analisa a variável idade, enquanto diferenças no perfil clínico (diabetes, síndrome metabólica, hipertensão arterial e dislipidemia) e bioquímico entre os grupos analisados não foram detectadas.

Em conjunto, nosso estudo traz novas perspectivas no entendimento do sistema renina-angiotensina no contexto da CAD, analisando o tecido adiposo branco perivascular e pericárdico. Portanto, novos estudos devem ser encorajados, pois propostas de outras investigações fisiológicas e moleculares poderão elucidar as lacunas aqui deixadas.

REFERÊNCIAS

1. Bray GA. Medical consequences of obesity. The Journal of clinical endocrinology and metabolism. 2004;89(6):2583-9.
2. World Health Organization. Obesity and overweight. 2018.
3. Hill JO, Wyatt HR, Peters JC. Energy balance and obesity. Circulation. 2012;126(1):126-32.
4. Walley AJ, Blakemore AIF, Froguel P. Genetics of obesity and the prediction of risk for health. Human Molecular Genetics. 2006;15(suppl_2):R124-R30.
5. Heo M, Allison DB, Faith MS, Zhu S, Fontaine KR. Obesity and quality of life: mediating effects of pain and comorbidities. Obesity research. 2003;11(2):209-16.
6. Tremmel M, Gerdtham UG, Nilsson PM, Saha S. Economic Burden of Obesity: A Systematic Literature Review. International journal of environmental research and public health. 2017;14(4).
7. Greenberg AS, Obin MS. Obesity and the role of adipose tissue in inflammation and metabolism. The American journal of clinical nutrition. 2006;83(2):461S-5S.
8. Coelho M, Oliveira T, Fernandes R. Biochemistry of adipose tissue: an endocrine organ. Archives of medical science : AMS. 2013;9(2):191-200.
9. Bluher M. Adipokines - removing road blocks to obesity and diabetes therapy. Molecular metabolism. 2014;3(3):230-40.
10. Bernlohr DA, Jenkins AE, Bennaars AA. Chapter 10 Adipose tissue and lipid metabolism. New Comprehensive Biochemistry. 36: Elsevier; 2002. p. 263-89.
11. Kershaw EE, Flier JS. Adipose tissue as an endocrine organ. The Journal of clinical endocrinology and metabolism. 2004;89(6):2548-56.
12. Fonseca-Alaniz MH, Takada J, Alonso-Vale MI, Lima FB. Adipose tissue as an endocrine organ: from theory to practice. Jornal de pediatria. 2007;83(5 Suppl):S192-203.
13. Saely CH, Geiger K, Drexel H. Brown versus white adipose tissue: a mini-review. Gerontology. 2012;58(1):15-23.
14. Costa JV, Duarte JS. [Adipose tissue and adipokines]. Acta medica portuguesa. 2006;19(3):251-6.
15. Fonseca-Alaniz MH, Takada J, Alonso-Vale MIC, Lima FB. O tecido adiposo como centro regulador do metabolismo. Arquivos Brasileiros de Endocrinologia & Metabologia. 2006;50:216-29.
16. Kiess W, Petzold S, Topfer M, Garten A, Bluher S, Kapellen T, et al. Adipocytes and adipose tissue. Best practice & research Clinical endocrinology & metabolism. 2008;22(1):135-53.
17. Ordovas JM, Corella D. Metabolic syndrome pathophysiology: the role of adipose tissue. Kidney international Supplement. 2008(111):S10-4.
18. Wronska A, Kmiec Z. Structural and biochemical characteristics of various white adipose tissue depots. Acta physiologica. 2012;205(2):194-208.
19. Salazar J, Luzardo E, Mejias JC, Rojas J, Ferreira A, Rivas-Rios JR, et al. Epicardial Fat: Physiological, Pathological, and Therapeutic Implications. Cardiology research and practice. 2016;2016:1291537.
20. Berg AH, Scherer PE. Adipose tissue, inflammation, and cardiovascular disease. Circulation research. 2005;96(9):939-49.
21. Ikeoka D, Mader JK, Pieber TR. Adipose tissue, inflammation and cardiovascular disease. Revista da Associação Médica Brasileira. 2010;56:116-21.
22. Oikonomou EK, Antoniades C. The role of adipose tissue in cardiovascular health and disease. Nature reviews Cardiology. 2018.

23. DeClercq V, Taylor C, Zahradka P. Adipose tissue: the link between obesity and cardiovascular disease. Cardiovascular & hematological disorders drug targets. 2008;8(3):228-37.
24. Sacks HS, Fain JN. Human epicardial adipose tissue: a review. American heart journal. 2007;153(6):907-17.
25. Moore KL, Persaud TVN. The developing human. Clinically oriented embryology. 7 ed. Philadelphia: Saunders; 2003.
26. Taguchi R, Takasu J, Itani Y, Yamamoto R, Yokoyama K, Watanabe S, et al. Pericardial fat accumulation in men as a risk factor for coronary artery disease. Atherosclerosis. 2001;157(1):203-9.
27. Cheung L, Gertow J, Werngren O, Folkersen L, Petrovic N, Nedergaard J, et al. Human mediastinal adipose tissue displays certain characteristics of brown fat. Nutrition & diabetes. 2013;3:e66.
28. Iacobellis G, Corradi D, Sharma AM. Epicardial adipose tissue: anatomic, biomolecular and clinical relationships with the heart. Nature clinical practice Cardiovascular medicine. 2005;2(10):536-43.
29. Fantuzzi G, Mazzone T. Adipose tissue and atherosclerosis: exploring the connection. Arteriosclerosis, thrombosis, and vascular biology. 2007;27(5):996-1003.
30. Huang G, Wang D, Zeb I, Budoff MJ, Harman SM, Miller V, et al. Intra-thoracic fat, cardiometabolic risk factors, and subclinical cardiovascular disease in healthy, recently menopausal women screened for the Kronos Early Estrogen Prevention Study (KEEPS). Atherosclerosis. 2012;221(1):198-205.
31. Tadros TM, Massaro JM, Rosito GA, Hoffmann U, Vasan RS, Larson MG, et al. Pericardial fat volume correlates with inflammatory markers: the Framingham Heart Study. Obesity. 2010;18(5):1039-45.
32. Sironi AM, Petz R, De Marchi D, Buzzigoli E, Ciociaro D, Positano V, et al. Impact of increased visceral and cardiac fat on cardiometabolic risk and disease. Diabetic medicine : a journal of the British Diabetic Association. 2012;29(5):622-7.
33. Rosito GA, Massaro JM, Hoffmann U, Ruberg FL, Mahabadi AA, Vasan RS, et al. Pericardial fat, visceral abdominal fat, cardiovascular disease risk factors, and vascular calcification in a community-based sample: the Framingham Heart Study. Circulation. 2008;117(5):605-13.
34. Sicari R, Sironi AM, Petz R, Frassi F, Chubuchny V, De Marchi D, et al. Pericardial rather than epicardial fat is a cardiometabolic risk marker: an MRI vs echo study. Journal of the American Society of Echocardiography : official publication of the American Society of Echocardiography. 2011;24(10):1156-62.
35. Wheeler GL, Shi R, Beck SR, Langefeld CD, Lenchik L, Wagenknecht LE, et al. Pericardial and visceral adipose tissues measured volumetrically with computed tomography are highly associated in type 2 diabetic families. Investigative radiology. 2005;40(2):97-101.
36. Meijer RI, Serne EH, Smulders YM, van Hinsbergh VW, Yudkin JS, Eringa EC. Perivascular adipose tissue and its role in type 2 diabetes and cardiovascular disease. Current diabetes reports. 2011;11(3):211-7.
37. Tang W, Zeve D, Suh JM, Bosnakovski D, Kyba M, Hammer RE, et al. White fat progenitor cells reside in the adipose vasculature. Science. 2008;322(5901):583-6.
38. Iacobellis G, Ribaudo MC, Assael F, Vecci E, Tiberti C, Zappaterreno A, et al. Echocardiographic epicardial adipose tissue is related to anthropometric and clinical parameters of metabolic syndrome: a new indicator of cardiovascular risk. The Journal of clinical endocrinology and metabolism. 2003;88(11):5163-8.

39. Rittig K, Staib K, Machann J, Bottcher M, Peter A, Schick F, et al. Perivascular fatty tissue at the brachial artery is linked to insulin resistance but not to local endothelial dysfunction. Diabetologia. 2008;51(11):2093-9.
40. Ouwens DM, Sell H, Greulich S, Eckel J. The role of epicardial and perivascular adipose tissue in the pathophysiology of cardiovascular disease. Journal of cellular and molecular medicine. 2010;14(9):2223-34.
41. Omar A, Chatterjee TK, Tang Y, Hui DY, Weintraub NL. Proinflammatory phenotype of perivascular adipocytes. Arteriosclerosis, thrombosis, and vascular biology. 2014;34(8):1631-6.
42. Tilg H, Moschen AR. Adipocytokines: mediators linking adipose tissue, inflammation and immunity. Nature reviews Immunology. 2006;6(10):772-83.
43. Police SB, Thatcher SE, Charnigo R, Daugherty A, Cassis LA. Obesity promotes inflammation in periaortic adipose tissue and angiotensin II-induced abdominal aortic aneurysm formation. Arteriosclerosis, thrombosis, and vascular biology. 2009;29(10):1458-64.
44. Feldon SE, O'Loughlin C W, Ray DM, Landskroner-Eiger S, Seweryniak KE, Phipps RP. Activated human T lymphocytes express cyclooxygenase-2 and produce proadipogenic prostaglandins that drive human orbital fibroblast differentiation to adipocytes. The American journal of pathology. 2006;169(4):1183-93.
45. Tsuruda T, Kato J, Hatakeyama K, Kojima K, Yano M, Yano Y, et al. Adventitial mast cells contribute to pathogenesis in the progression of abdominal aortic aneurysm. Circulation research. 2008;102(11):1368-77.
46. Chatterjee TK, Stoll LL, Denning GM, Harrelson A, Blomkalns AL, Idelman G, et al. Proinflammatory phenotype of perivascular adipocytes: influence of high-fat feeding. Circulation research. 2009;104(4):541-9.
47. Greenstein AS, Khavandi K, Withers SB, Sonoyama K, Clancy O, Jeziorska M, et al. Local inflammation and hypoxia abolish the protective anticontractile properties of perivascular fat in obese patients. Circulation. 2009;119(12):1661-70.
48. Henrichot E, Juge-Aubry CE, Pernin A, Pache JC, Velebit V, Dayer JM, et al. Production of chemokines by perivascular adipose tissue: a role in the pathogenesis of atherosclerosis? Arteriosclerosis, thrombosis, and vascular biology. 2005;25(12):2594-9.
49. Bahia LR, Rosa RS, Santos RD, Araujo DV. Estimated costs of hospitalization due to coronary artery disease attributable to familial hypercholesterolemia in the Brazilian public health system. Archives of Endocrinology and Metabolism. 2018;62:303-8.
50. Bhatia SK. Coronary Artery Disease. Biomaterials for Clinical Applications. . New York: Springer
2010.
51. Ashley EA, Niebauer J. Cardiology explained. London: Remedica; 2004.
52. Glasser SP, Selwyn AP, Ganz P. Atherosclerosis: risk factors and the vascular endothelium. American heart journal. 1996;131(2):379-84.
53. Lovren F, Teoh H, Verma S. Obesity and atherosclerosis: mechanistic insights. The Canadian journal of cardiology. 2015;31(2):177-83.
54. Skeoch S, Bruce IN. Atherosclerosis in rheumatoid arthritis: is it all about inflammation? Nature Reviews Rheumatology. 2015;11:390.
55. McGill HC, Jr., Herderick EE, McMahan CA, Zieske AW, Malcolm GT, Tracy RE, et al. Atherosclerosis in youth. Minerva pediatrica. 2002;54(5):437-47.
56. Hajjar DP, Gotto AM, Jr. Biological relevance of inflammation and oxidative stress in the pathogenesis of arterial diseases. The American journal of pathology. 2013;182(5):1474-81.

57. Engeli S, Negrel R, Sharma AM. Physiology and pathophysiology of the adipose tissue renin-angiotensin system. Hypertension. 2000;35(6):1270-7.
58. Li YC, Kong J, Wei M, Chen ZF, Liu SQ, Cao LP. 1,25-Dihydroxyvitamin D(3) is a negative endocrine regulator of the renin-angiotensin system. The Journal of clinical investigation. 2002;110(2):229-38.
59. Grace JA, Herath CB, Mak KY, Burrell LM, Angus PW. Update on new aspects of the renin-angiotensin system in liver disease: clinical implications and new therapeutic options. Clinical science. 2012;123(4):225-39.
60. Thatcher S, Yiannikouris F, Gupte M, Cassis L. The adipose renin-angiotensin system: role in cardiovascular disease. Molecular and cellular endocrinology. 2009;302(2):111-7.
61. Galvez-Prieto B, Bolbrinker J, Stucchi P, de Las Heras AI, Merino B, Arribas S, et al. Comparative expression analysis of the renin-angiotensin system components between white and brown perivascular adipose tissue. The Journal of endocrinology. 2008;197(1):55-64.
62. Engeli S, Schling P, Gorzelniak K, Boschmann M, Janke J, Ailhaud G, et al. The adipose-tissue renin-angiotensin-aldosterone system: role in the metabolic syndrome? The international journal of biochemistry & cell biology. 2003;35(6):807-25.
63. Szasz T, Bomfim GF, Webb RC. The influence of perivascular adipose tissue on vascular homeostasis. Vascular health and risk management. 2013;9:105-16.
64. Eringa EC, Bakker W, van Hinsbergh VW. Paracrine regulation of vascular tone, inflammation and insulin sensitivity by perivascular adipose tissue. Vascular pharmacology. 2012;56(5-6):204-9.
65. Bailey-Downs LC, Tucsek Z, Toth P, Sosnowska D, Gautam T, Sonntag WE, et al. Aging exacerbates obesity-induced oxidative stress and inflammation in perivascular adipose tissue in mice: a paracrine mechanism contributing to vascular redox dysregulation and inflammation. The journals of gerontology Series A, Biological sciences and medical sciences. 2013;68(7):780-92.
66. Campia U, Tesauro M, Cardillo C. Human obesity and endothelium-dependent responsiveness. British journal of pharmacology. 2012;165(3):561-73.
67. Aroor AR, McKarns S, Demarco VG, Jia G, Sowers JR. Maladaptive immune and inflammatory pathways lead to cardiovascular insulin resistance. Metabolism: clinical and experimental. 2013;62(11):1543-52.
68. Cassis LA, Police SB, Yiannikouris F, Thatcher SE. Local adipose tissue renin-angiotensin system. Current hypertension reports. 2008;10(2):93-8.
69. Thatcher S, Yiannikouris F, Gupte M, Cassis L. The adipose renin–angiotensin system: Role in cardiovascular disease. Molecular and cellular endocrinology. 2009;302(2):111-7.

ANEXOS

ANEXO A – Parecer do Comitê de Ética e Pesquisa

UNIVERSIDADE ESTADUAL DE MONTES CLAROS - UNIMONTES

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Associação entre dados clínicos, de composição corporal e estado nutricional de pacientes com doença cardiovascular avançada com a expressão de biomoléculas no tecido adiposo.

Pesquisador: Sérgio Henrique Sousa Santos

Área Temática:

Versão: 2

CAAE: 66566117.8.0000.5146

Instituição Proponente: Universidade Estadual de Montes Claros - UNIMONTES

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 2.073.219

Apresentação do Projeto:

O presente projeto se caracteriza como um estudo transversal, prospectivo, analítico e de abordagem predominantemente quantitativa.Serão utilizadas amostras biológicas de tecido adiposo e soro em associação à dados clínicos, oriundos de pacientes com doença cardiovascular avançada, submetidos à cirurgia de revascularização miocárdica, armazenadas no Biobanco de Materiais Biológicos da Universidade Estadual de Montes Claros. Serão analisados contagem total de hemácias, leucócitos e dos níveis plasmáticos de glicose, hemoglobina glicosilada A1C,colesterol total e frações, triglicerídeos, ácido úrico,ureia, creatinina, albumina, proteína C reativa quantitativa, insulina, aspartatoaminostransferase, alanina aminotransferase e transferrina, ferritina. Além da expressão plasmática de adiponectina, leptina, TNF-alfa, IL-6, ECA, ECA2 e AGT. Á nível
tecidual, serão realizadas análises histológicas, e de imunohistoquímica, visando definir estrutura tecidual e expressão de moléculas proteicas específicas. Será ainda mensurada a expressão tecidual de mRNA dos seguintes marcadores: Enzima Conversora de Angiotensina (ACE), Enzima Conversora de Angiotensina 2 (ACE-2), Angiotensina II (Ang II), Receptor Mas (Mas), Receptor AT1 (AT1), Fator de Necrose Tumoral (TNF)-, Interleucina -6 (IL-6), Metaloproteinases de matriz (MMP), Proteína desacopladora 1 (UCP1), Proteína 16 contendo o domínio PR (PRDM16), Receptor Alfa

Endereço: Av.Dr Rui Braga s/n-Camp Univers Profº Darcy Rib
Bairro: Vila Mauricéia **CEP:** 39.401-089
UF: MG **Município:** MONTES CLAROS
Telefone: (38)3229-8180 **Fax:** (38)3229-8103 **E-mail:** smelocosta@gmail.com

Ativado por Proliferador de Peroxissoma (PGC1-a), Proteína óssea morfogenética 7 (BMP-7), Sirtuínas (1 a 14), beta3-AR, beta1-AR e
-actina/GAPDH como controles endógenos. Ainda nas amostras de tecido adiposo coletadas, análises da atividade de enzimas relacionadas ao stress oxidativo serão realizadas. Serão realizadas ainda bateladas de cultura de adipócitos, onde inibidores específicos do sistema reninaangiotensina, sirtuínas e termogênese serão aplicados, seguidos da mensuração dos marcadores respectivos das vias em análise no momento (Sirtuínas 1-7, PRDM16, UCP1, BMP-7, Cidea, AGT, ACE, ACE2, ANG-(1-7), AT1, Mas). Todos os dados coletados a partir da investigação das variáveis sócio-demográficas, clínicas e das análises de laudos de exames complementares que caracterizam a doença cardiovascular avançada serão digitalizados e posteriormente analisados

Objetivo da Pesquisa:

Analisar a influência de dados clínicos e da expressão de biomoléculas dos tecidos adiposos pericárdico e epicárdico nos pacientes com doença cardiovascular avançada submetidos à cirurgia de revascularização miocárdica.

Avaliação dos Riscos e Benefícios:

Segundo os pesquisadores Riscos:

Os riscos quanto à doação do material estão relacionados ao tipo de procedimento realizado pela equipe médica, necessário para diagnóstico e tratamento que deverão ser claramente esclarecidos para você pela equipe. Sendo que a coleta de material para o Biobanco envolve apenas o excedente material biológico proveniente desse procedimento médico. Os riscos relacionados à coleta dos dados clínicos, de composição corporal e nutricional são minimizados com o treinamento da equipe técnica responsável por aplicar os questionários.

Benefícios:

Quanto aos benefícios, a doação do material poderá favorecer a realização de pesquisas que buscam um melhor entendimento e possibilidade de controle e tratamento das doenças, visando melhor entender as causas e mecanismos das doenças.

Comentários e Considerações sobre a Pesquisa:

Pesquisa relevante na área de doenças cardiovasculares, que segundo o pesquisador todas as

UNIVERSIDADE ESTADUAL DE MONTES CLAROS - UNIMONTES

informações a serem adotadas neste estudo, como os dados clínicos, a composição corporal e o estado nutricional dos pacientes são oriundos de arquivos do Biobanco de materiais biológicos do Norte de Minas Gerais, além do excedente material biológico doado a esse setor.

Considerações sobre os Termos de apresentação obrigatória:

Adequados.

Recomendações:

Apresentação de relatório final por meio da plataforma Brasil, em "enviar notificação".

Conclusões ou Pendências e Lista de Inadequações:

Aprovado.

Considerações Finais a critério do CEP:

O projeto respeita os preceitos éticos da pesquisa em seres humanos, sendo assim somos favoráveis à aprovação do mesmo.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_889813.pdf	06/05/2017 16:19:09		Aceito
Outros	INFORMACOES_CLINICAS_BIOBANCO.pdf	06/05/2017 16:18:24	Sérgio Henrique Sousa Santos	Aceito
Projeto Detalhado / Brochura Investigador	PROJETO_DETALHADO.pdf	06/05/2017 16:18:04	Sérgio Henrique Sousa Santos	Aceito
Outros	PARECER_CONEP_BIOBANCO.pdf	30/03/2017 14:30:40	Sérgio Henrique Sousa Santos	Aceito
Outros	DECLARACAO.pdf	30/03/2017 14:30:12	Sérgio Henrique Sousa Santos	Aceito
Outros	JUSTIFICATIVA.pdf	30/03/2017 14:28:48	Sérgio Henrique Sousa Santos	Aceito
Folha de Rosto	Folha_de_rosto.pdf	30/03/2017 14:06:32	Sérgio Henrique Sousa Santos	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE.pdf	27/03/2017 10:53:44	Sérgio Henrique Sousa Santos	Aceito

Endereço: Av.Dr Rui Braga s/n-Camp Univers Profº Darcy Rib
Bairro: Vila Mauricéia **CEP:** 39.401-089
UF: MG **Município:** MONTES CLAROS
Telefone: (38)3229-8180 **Fax:** (38)3229-8103 **E-mail:** smelocosta@gmail.com

UNIVERSIDADE ESTADUAL DE MONTES CLAROS - UNIMONTES

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

MONTES CLAROS, 19 de Maio de 2017

Assinado por:
SIMONE DE MELO COSTA
(Coordenador)

Endereço: Av.Dr Rui Braga s/n-Camp Univers Profº Darcy Rib
Bairro: Vila Mauricéia **CEP:** 39.401-089
UF: MG **Município:** MONTES CLAROS
Telefone: (38)3229-8180 **Fax:** (38)3229-8103 **E-mail:** smelocosta@gmail.com

ANEXO B – Normas para publicação no periódico *Life Sciences*

LIFE SCIENCES

DESCRIPTION

Life Sciences is an international journal publishing articles that emphasize the **molecular**, **cellular**, and **functional basis of therapy**. The journal emphasizes the understanding of mechanism that is relevant to all aspects of human disease and translation to patients. All articles are rigorously reviewed.

The Journal favors publication of full-length papers where modern scientific technologies are used to explain **molecular**, **cellular** and **physiological mechanisms**. Articles that merely report observations are rarely accepted. Recommendations from the Declaration of Helsinki or NIH guidelines for care and use of laboratory animals must be adhered to. Articles should be written at a level accessible to readers who are non-specialists in the topic of the article themselves, but who are interested in the research. The Journal welcomes reviews on topics of wide interest to investigators in the **life sciences**. We particularly encourage submission of brief, focused reviews containing high- quality artwork and require the use of mechanistic summary diagrams.

Manuscripts should present novel preclinical findings addressing questions of **biological significance** to **human disease**. Studies that fail to do so may be rejected without review. Quantitative conclusions must be based on truly quantitative methods. *Life Sciences* does not publish work on the actions of biological extracts of unknown chemical composition. Compounds studied must be of known chemical structure and concentration. The study must be reproducible; materials used must be available to other researchers so they can repeat the experiment. Clinical studies may be considered if they expand understanding of mechanism, but the journal does not encourage clinical trial reports.

Four common reasons for rejection include: out of scope (the manuscript does not conform to the goal of identification of mechanisms related to therapy for human disease); too preliminary (manuscript is based on a limited amount of experimental data diminishing significance); lack of novelty (manuscript is well done but does not address a significant question); unidentified structure (actions of biological extracts of unknown chemical composition).

EDITORIAL BOARD

GUIDE FOR AUTHORS

Your Paper Your Way

We now differentiate between the requirements for new and revised submissions. You may choose to submit your manuscript as a single Word or PDF file to be used in the refereeing process. Only when your paper is at the revision stage, will you be requested to put your paper in to a 'correct format' for acceptance and provide the items required for the publication of your article.
To find out more, please visit the Preparation section below.

INTRODUCTION

Life Sciences is an international journal publishing articles that emphasize the molecular, cellular, and functional basis of therapy. All articles are rigorously reviewed. The Journal favors publication of full-length papers where modern scientific technologies are used to explain molecular, cellular and physiological mechanisms. Articles that merely report observations are rarely accepted. Articles should be written at a level accessible to readers who are non-specialists in the topic of the article themselves, but who are interested in the research.
The Journal welcomes reviews on topics of wide interest to investigators in the life sciences. We particularly encourage submission of focused reviews containing high-quality artwork and mechanistic diagrams.

IMPORTANT INFORMATION

- Submission of a paper will be held to imply that the manuscript contains original unpublished work and is not being submitted for publication elsewhere.
- Manuscripts should present novel findings addressing significant biological questions. Studies that fail to do so may be rejected without review.
- Quantitative conclusions must be based on truly quantitative methods.
- Life Sciences does not publish work on the actions of biological extracts of unknown chemical composition. Compounds studied must be of known chemical structure and concentration.
- The study must be reproducible; materials used must be available to other researchers so they can repeat the experiment.

For more details on how to write a world class paper, please visit our Pharmacology Author Resources page.
Please include word count and figure/table count on the cover page of your manuscript.

Authors are encouraged to submit video material or animation sequences to support and enhance your scientific research. For more information please see the paragraph on video data below.

Types of article

- Original research articles
- Reviews

Submission checklist
You can use this list to carry out a final check of your submission before you send it to the journal for review. Please check the relevant section in this Guide for Authors for more details.

Ensure that the following items are present:

One author has been designated as the corresponding author with contact details:
- E-mail address
- Full postal address

All necessary files have been uploaded:
Manuscript:
- Include keywords
- All figures (include relevant captions)
- All tables (including titles, description, footnotes)
- Ensure all figure and table citations in the text match the files provided
- Indicate clearly if color should be used for any figures in print Graphical Abstracts / Highlights files (where applicable) Supplemental files (where applicable)

Further considerations
- Manuscript has been 'spell checked' and 'grammar checked'
- All references mentioned in the Reference List are cited in the text, and vice versa
- Permission has been obtained for use of copyrighted material from other sources (including the Internet)
- A competing interests statement is provided, even if the authors have no competing interests to declare
- Journal policies detailed in this guide have been reviewed
- Referee suggestions and contact details provided, based on journal requirements

For further information, visit our Support Center.

BEFORE YOU BEGIN

Ethics in publishing
Please see our information pages on Ethics in publishing and Ethical guidelines for journal publication.

Studies in humans and animals
If the work involves the use of human subjects, the author should ensure that the work described has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. The manuscript should be in line with the Recommendations for the Conduct, Reporting, Editing and Publication of Scholarly Work in Medical Journals and aim for the inclusion of representative human populations (sex, age and ethnicity) as per those recommendations. The terms sex and gender should be used correctly.

Authors should include a statement in the manuscript that informed consent was obtained for experimentation with human subjects. The privacy rights of human subjects must always be observed.

All animal experiments should comply with the ARRIVE guidelines and should be carried out in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments, or the National Institutes of

Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978) and the authors should clearly indicate in the manuscript that such guidelines have been followed. The sex of animals must be indicated, and where appropriate, the influence (or association) of sex on the results of the study.

Conflict of Interest Policy
The Journal requires full disclosure of all potential conflicts of interest. At the end of the manuscript text, under a subheading "Conflict of Interest statement", all authors must disclose any financial and personal relationships with other people or organisations that could inappropriately influence (bias) their work. If there are no conflicts of interest, the authors should state: "The authors declare that there are no conflicts of interest." See also http://www.elsevier.com/conflictsofinterest. A signed Conflict of Interests Policy Form is required upon submission. The corresponding author is responsible for completing the form, and signing it on behalf of all authors.
Submission declaration and verification

Submission of an article implies that the work described has not been published previously (except in the form of an abstract, a published lecture or academic thesis, see 'Multiple, redundant or concurrent publication' for more information), that it is not under consideration for publication elsewhere, that its publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out, and that, if accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically without the written consent of the copyright- holder. To verify originality, your article may be checked by the originality detection service Crossref Similarity Check.

Use of inclusive language
Inclusive language acknowledges diversity, conveys respect to all people, is sensitive to differences, and promotes equal opportunities. Articles should make no assumptions about the beliefs or commitments of any reader, should contain nothing which might imply that one individual is superior to another on the grounds of race, sex, culture or any other characteristic, and should use inclusive language throughout. Authors should ensure that writing is free from bias, for instance by using 'he or she', 'his/her' instead of 'he' or 'his', and by making use of job titles that are free of stereotyping (e.g. 'chairperson' instead of 'chairman' and 'flight attendant' instead of 'stewardess').

Author contributions
For transparency, we encourage authors to submit an author statement file outlining their individual contributions to the paper using the relevant CRediT roles: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Software; Supervision; Validation; Visualization; Roles/Writing - original draft; Writing - review & editing. Authorship statements should be formatted with the names of authors first and CRediT role(s) following. More details and an example

Authorship
All authors listed on your paper must have made significant contributions to the study. To ensure clarity, you are required upon submission to enter the specific details of each author's contribution, which must substantiate the inclusion of each person on the manuscript. This information is required to be filled in on the Conflict of Interests Policy and Author Statement Form.

Changes to authorship
Authors are expected to consider carefully the list and order of authors before submitting their manuscript and provide the definitive list of authors at the time of the original submission. Any addition, deletion or rearrangement of author names in the authorship list should be made only before the manuscript has been accepted and only if approved by the journal Editor. To request such a change, the Editor must receive the following from the corresponding author: (a) the reason for the change in author list and (b) written confirmation (e-mail, letter) from all authors that they agree with the addition, removal or rearrangement. In the case of addition or removal of authors, this includes confirmation from the author being added or removed.
Only in exceptional circumstances will the Editor consider the addition, deletion or rearrangement of authors after the manuscript has been accepted. While the Editor considers the request, publication of the manuscript will be suspended. If the manuscript has already been published in an online issue, any requests approved by the Editor will result in a corrigendum.

Copyright
Upon acceptance of an article, authors will be asked to complete a 'Journal Publishing Agreement' (see more information on this). An e-mail will be sent to the corresponding author confirming receipt of the manuscript together with a 'Journal Publishing Agreement' form or a link to the online version of this agreement.

Subscribers may reproduce tables of contents or prepare lists of articles including abstracts for internal circulation within their institutions. Permission of the Publisher is required for resale or distribution outside the institution and for all other derivative works, including compilations and translations. If excerpts from other copyrighted works are included, the author(s) must obtain written permission from the copyright owners and credit the source(s) in the article. Elsevier has preprinted forms for use by authors in these cases.

For gold open access articles: Upon acceptance of an article, authors will be asked to complete an 'Exclusive License Agreement' (more information). Permitted third party reuse of gold open access articles is determined by the author's choice of user license.

Author rights
As an author you (or your employer or institution) have certain rights to reuse your work. More information.
Elsevier supports responsible sharing
Find out how you can share your research published in Elsevier journals.

Role of the funding source
You are requested to identify who provided financial support for the conduct of the research and/or preparation of the article and to briefly describe the role of the sponsor(s), if any, in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication. If the funding source(s) had no such involvement then this should be stated.
Funding body agreements and policies
Elsevier has established a number of agreements with funding bodies which allow authors to comply with their funder's open access policies. Some funding bodies will reimburse the

author for the gold open access publication fee. Details of existing agreements are available online.

Open access
This journal offers authors a choice in publishing their research:

Subscription
- Articles are made available to subscribers as well as developing countries and patient groups through our universal access programs.
- No open access publication fee payable by authors.
- The Author is entitled to post the accepted manuscript in their institution's repository and make this public after an embargo period (known as green Open Access). The published journal article cannot be shared publicly, for example on ResearchGate or Academia.edu, to ensure the sustainability of peer- reviewed research in journal publications. The embargo period for this journal can be found below. Gold open access
- Articles are freely available to both subscribers and the wider public with permitted reuse.
- A gold open access publication fee is payable by authors or on their behalf, e.g. by their research funder or institution.

Regardless of how you choose to publish your article, the journal will apply the same peer review criteria and acceptance standards.

For gold open access articles, permitted third party (re)use is defined by the following Creative Commons user licenses:

Creative Commons Attribution (CC BY)
Lets others distribute and copy the article, create extracts, abstracts, and other revised versions, adaptations or derivative works of or from an article (such as a translation), include in a collective work (such as an anthology), text or data mine the article, even for commercial purposes, as long as they credit the author(s), do not represent the author as endorsing their adaptation of the article, and do not modify the article in such a way as to damage the author's honor or reputation.

Creative Commons Attribution-NonCommercial-NoDerivs (CC BY-NC-ND)
For non-commercial purposes, lets others distribute and copy the article, and to include in a collective work (such as an anthology), as long as they credit the author(s) and provided they do not alter or modify the article.
The gold open access publication fee for this journal is USD 3300, excluding taxes. Learn more about Elsevier's pricing policy: https://www.elsevier.com/openaccesspricing.

Green open access
Authors can share their research in a variety of different ways and Elsevier has a number of green open access options available. We recommend authors see our green open access page for further information. Authors can also self-archive their manuscripts immediately and enable public access from their institution's repository after an embargo period. This is the version that has been accepted for publication and which typically includes author-incorporated changes suggested during submission, peer review and in editor-author

communications. Embargo period: For subscription articles, an appropriate amount of time is needed for journals to deliver value to subscribing customers before an article becomes freely available to the public. This is the embargo period and it begins from the date the article is formally published online in its final and fully citable form. Find out more.
This journal has an embargo period of 12 months.

Elsevier Researcher Academy
Researcher Academy is a free e-learning platform designed to support early and mid-career researchers throughout their research journey. The "Learn" environment at Researcher Academy offers several interactive modules, webinars, downloadable guides and resources to guide you through the process of writing for research and going through peer review. Feel free to use these free resources to improve your submission and navigate the publication process with ease.

Language (usage and editing services)
Please write your text in good English (American or British usage is accepted, but not a mixture of these). Authors who feel their English language manuscript may require editing to eliminate possible grammatical or spelling errors and to conform to correct scientific English may wish to use the English Language Editing service available from Elsevier's WebShop.

Submission
Submission to this journal proceeds totally online. Use the following guidelines to prepare your article. Via the homepage of this journal (http://www.elsevier.com/journals) you will be guided stepwise through the creation and uploading of the various files. The system automatically converts source files to a single Adobe Acrobat PDF version of the article, which is used in the peer-review process. Please note that even though manuscript source files are converted to PDF at submission for the review process, these source files are needed for further processing after acceptance. All correspondence, including notification of the Editor's decision and requests for revision, takes place by e-mail and via the author's page in EES, removing the need for a hard-copy paper trail.

Specific queries can be directed to lifesci@elsevier.com.

Referees
To expedite the review process, authors must submit names of 4 - 6 individuals who are qualified to review their work. Include the email address of each potential referee, as much contact information as possible, and why you feel this person is competent to review your work. You should not have collaborated with the suggested reviewers at any time in the past five years. In our effort to enhance global perspective and communication of science, these individuals should be associated with institutions from as many different regions as possible (Europe, North America, Asia, etc.). Exception: Symposium submissions which have been previously reviewed and approved by their Organizing Committee.

PREPARATION

NEW SUBMISSIONS
Submission to this journal proceeds totally online and you will be guided stepwise through the creation and uploading of your files. The system automatically converts your files to a single PDF file, which is used in the peer-review process.

As part of the Your Paper Your Way service, you may choose to submit your manuscript as a single file to be used in the refereeing process. This can be a PDF file or a Word document, in any format or lay- out that can be used by referees to evaluate your manuscript. It should contain high enough quality figures for refereeing. If you prefer to do so, you may still provide all or some of the source files at the initial submission. Please note that individual figure files larger than 10 MB must be uploaded separately.

References
There are no strict requirements on reference formatting at submission. References can be in any style or format as long as the style is consistent. Where applicable, author(s) name(s), journal title/ book title, chapter title/article title, year of publication, volume number/book chapter and the article number or pagination must be present. Use of DOI is highly encouraged. The reference style used by the journal will be applied to the accepted article by Elsevier at the proof stage. Note that missing data will be highlighted at proof stage for the author to correct.

Formatting requirements
There are no strict formatting requirements but all manuscripts must contain the essential elements needed to convey your manuscript, for example Abstract, Keywords, Introduction, Materials and Methods, Results, Conclusions, Artwork and Tables with Captions.
If your article includes any Videos and/or other Supplementary material, this should be included in your initial submission for peer review purposes.
Divide the article into clearly defined sections.
Figures and tables embedded in text

Please ensure the figures and the tables included in the single file are placed next to the relevant text in the manuscript, rather than at the bottom or the top of the file. The corresponding caption should be placed directly below the figure or table.

Peer review
This journal operates a double blind review process. All contributions will be initially assessed by the editor for suitability for the journal. Papers deemed suitable are then typically sent to a minimum of two independent expert reviewers to assess the scientific quality of the paper. The Editor is responsible for the final decision regarding acceptance or rejection of articles. The Editor's decision is final. More information on types of peer review.

Double-blind review
This journal uses double-blind review, which means the identities of the authors are concealed from the reviewers, and vice versa. More information is available on our website. To facilitate this, please include the following separately:
Title page (with author details): This should include the title, authors' names, affiliations, acknowledgements and any Declaration of Interest statement, and a complete address for the corresponding author including an e-mail address.
Blinded manuscript (no author details): The main body of the paper (including the references, figures, tables and any acknowledgements) should not include any identifying information, such as the authors' names or affiliations.

Language

Please write your text in good English (American or British usage is accepted, but not a mixture of these). For language assistance, please see Language Services, above. Use decimal points (not decimal commas); use a space for thousands (10 000 and above).

Use of word processing software
Regardless of the file format of the original submission, at revision you must provide us with an editable file of the entire article. Keep the layout of the text as simple as possible. Most formatting codes will be removed and replaced on processing the article. The electronic text should be prepared in a way very similar to that of conventional manuscripts (see also the Guide to Publishing with Elsevier). See also the section on Electronic artwork.
To avoid unnecessary errors you are strongly advised to use the 'spell-check' and 'grammar-check' functions of your word processor.

Organization of the manuscript
Beginning with the first page, present your manuscript in the order below:
1. Title: First letter capitalized, subsequent letters in lower case. Maximum length 150 characters including spaces. Avoid abbreviations.
2a. Names of all authors.
2b. Affiliations of all authors. If necessary, use superscripted lowercase letters after the author's name to distinguish affiliations.
3. Author to whom proofs and correspondence should be sent, including name, mailing address, telephone and fax numbers, and e-mail address.
4. A structured abstract has to be submitted for full length articles (not for reviews) of no more than 250 words.

Word limits: In **full papers**, individual sections should be no longer than Abstract 250 words, Introduction 500 words, Discussion 1500 words, Conclusion 150 words. Materials and Methods and Results sections should be concise but there is no formal word limit.

Headings: Papers must include the major headings Abstract, Introduction, Materials and Methods, Results, Discussion, Conclusion, Acknowledgments, and References. Include subheadings as appropriate. Review articles must contain Abstract and Introduction, with subsequent headings and subheadings as appropriate.

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

Material and methods
Provide sufficient details to allow the work to be reproduced by an independent researcher. Methods that are already published should be summarized, and indicated by a reference. If quoting directly from a previously published method, use quotation marks and also cite the source. Any modifications to existing methods should also be described.

Results
Results should be clear and concise.
Discussion
This should explore the significance of the results of the work, not repeat them. Avoid extensive citations and discussion of published literature.

Conclusions

Present the conclusions of the study in a short Conclusions section.
The Graphical Abstract is optional for research articles, but mandatory for reviews. GAs should summarize the contents of the article in a concise, pictorial form designed to capture the attention of a wide readership online. Graphical abstracts should be submitted as a separate file in the online submission system. Refer to the following website for more information http://www.elsevier.com/graphicalabstracts.

Abbreviations
Abbreviations must be explained the first time they are used, both in the Abstract and again in the main text.

Abbreviations used as names of cell lines do not need to be explained, but the species and tissue of origin should be made clear in text the first time the cell line is mentioned. Examples: "the human colonic adenocarcinoma cell line Caco-2" or "the porcine renal endothelial cell line LLC-PK1".

Acknowledgements
Collate acknowledgements in a separate section at the end of the article before the references and do not, therefore, include them on the title page, as a footnote to the title or otherwise. List here those individuals who provided help during the research (e.g., providing language help, writing assistance or proof reading the article, etc.).
Please note that funding information must appear under the Acknowledgments heading.
Formatting of funding sources
List funding sources in this standard way to facilitate compliance to funder's requirements:

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

It is not necessary to include detailed descriptions on the program or type of grants and awards. When funding is from a block grant or other resources available to a university, college, or other research institution, submit the name of the institute or organization that provided the funding.

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

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

Footnotes
Footnotes should be used sparingly. Number them consecutively throughout the article. Many word processors build footnotes into the text, and this feature may be used. Should this not be the case, indicate the position of footnotes in the text and present the footnotes themselves separately at the end of the article.

A detailed guide on electronic artwork is available.
You are urged to visit this site; some excerpts from the detailed information are given here.
Formats

Regardless of the application used, when your electronic artwork is finalized, please 'save as' or convert the images to one of the following formats (note the resolution requirements for line drawings, halftones, and line/halftone combinations given below):
EPS (or PDF): Vector drawings. Embed the font or save the text as 'graphics'.
TIFF (or JPG): Color or grayscale photographs (halftones): always use a minimum of 300 dpi.
TIFF (or JPG): Bitmapped line drawings: use a minimum of 1000 dpi.
TIFF (or JPG): Combinations bitmapped line/half-tone (color or grayscale): a minimum of 500 dpi is required.

Please do not:
- Supply files that are optimized for screen use (e.g., GIF, BMP, PICT, WPG); the resolution is too low.
- Supply files that are too low in resolution.
- Submit graphics that are disproportionately large for the content.

Color artwork
Please make sure that artwork files are in an acceptable format (TIFF (or JPEG), EPS (or PDF), or MS Office files) and with the correct resolution. If, together with your accepted article, you submit usable color figures then Elsevier will ensure, at no additional charge, that these figures will appear in color online (e.g., ScienceDirect and other sites) regardless of whether or not these illustrations are reproduced in color in the printed version. For color reproduction in print, you will receive information regarding the costs from Elsevier after receipt of your accepted article. Please indicate your preference for color: in print or online only. Further information on the preparation of electronic artwork.

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

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

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

A DOI is guaranteed never to change, so you can use it as a permanent link to any electronic article. An example of a citation using DOI for an article not yet in an issue is: VanDecar J.C.,

Russo R.M., James D.E., Ambeh W.B., Franke M. (2003). Aseismic continuation of the Lesser Antilles slab beneath northeastern Venezuela. Journal of Geophysical Research, https://doi.org/10.1029/2001JB000884. Please note the format of such citations should be in the same style as all other references in the paper.

Data references

This journal encourages you to cite underlying or relevant datasets in your manuscript by citing them in your text and including a data reference in your Reference List. Data references should include the following elements: author name(s), dataset title, data repository, version (where available), year, and global persistent identifier. Add [dataset] immediately before the reference so we can properly identify it as a data reference. The [dataset] identifier will not appear in your published article.

Reference management software

Most Elsevier journals have their reference template available in many of the most popular reference management software products. These include all products that support Citation Style Language styles, such as Mendeley and Zotero, as well as EndNote. Using the word processor plug-ins from these products, authors only need to select the appropriate journal template when preparing their article, after which citations and bibliographies will be automatically formatted in the journal's style. If no template is yet available for this journal, please follow the format of the sample references and citations as shown in this Guide. If you use reference management software, please ensure that you remove all field codes before submitting the electronic manuscript. More information on how to remove field codes.

Users of Mendeley Desktop can easily install the reference style for this journal by clicking the following link:
http://open.mendeley.com/use-citation-style/life-sciences
When preparing your manuscript, you will then be able to select this style using the Mendeley plug- ins for Microsoft Word or LibreOffice.

Reference formatting

There are no strict requirements on reference formatting at submission. References can be in any style or format as long as the style is consistent. Where applicable, author(s) name(s), journal title/ book title, chapter title/article title, year of publication, volume number/book chapter and the article number or pagination must be present. Use of DOI is highly encouraged. The reference style used by the journal will be applied to the accepted article by Elsevier at the proof stage. Note that missing data will be highlighted at proof stage for the author to correct. If you do wish to format the references yourself they should be arranged according to the following examples:

Reference style

Text: Indicate references by number(s) in square brackets in line with the text. The actual authors can be referred to, but the reference number(s) must always be given.
Example: '..... as demonstrated [3,6]. Barnaby and Jones [8] obtained a different result ....'
List: Number the references (numbers in square brackets) in the list in the order in which they appear in the text.
Examples:
Reference to a journal publication:
[1] J. van der Geer, J.A.J. Hanraads, R.A. Lupton, The art of writing a scientific article, J. Sci. Commun. 163 (2010) 51–59. https://doi.org/10.1016/j.Sc.2010.00372.

Reference to a journal publication with an article number:
[2] Van der Geer, J., Hanraads, J.A.J., Lupton, R.A., 2018. The art of writing a scientific article. Heliyon. 19, e00205. https://doi.org/10.1016/j.heliyon.2018.e00205.
Reference to a book:
[3] W. Strunk Jr., E.B. White, The Elements of Style, fourth ed., Longman, New York, 2000. Reference to a chapter in an edited book:
[4] G.R. Mettam, L.B. Adams, How to prepare an electronic version of your article, in: B.S. Jones, R.Z. Smith (Eds.), Introduction to the Electronic Age, E-Publishing Inc., New York, 2009, pp. 281–304. Reference to a website:
[5] Cancer Research UK, Cancer statistics reports for the UK. http://www.cancerresearchuk.org/ aboutcancer/statistics/cancerstatsreport/, 2003 (accessed 13 March 2003).
Reference to a dataset:
[dataset] [6] M. Oguro, S. Imahiro, S. Saito, T. Nakashizuka, Mortality data for Japanese oak wilt disease and surrounding forest compositions, Mendeley Data, v1, 2015. https://doi.org/10.17632/ xwj98nb39r.1.

Video
Elsevier accepts video material and animation sequences to support and enhance your scientific research. Authors who have video or animation files that they wish to submit with their article are strongly encouraged to include links to these within the body of the article. This can be done in the same way as a figure or table by referring to the video or animation content and noting in the body text where it should be placed. All submitted files should be properly labeled so that they directly relate to the video file's content. . In order to ensure that your video or animation material is directly usable, please provide the file in one of our recommended file formats with a preferred maximum size of 150 MB per file, 1 GB in total. Video and animation files supplied will be published online in the electronic version of your article in Elsevier Web products, including ScienceDirect. Please supply 'stills' with your files: you can choose any frame from the video or animation or make a separate image. These will be used instead of standard icons and will personalize the link to your video data. For more detailed instructions please visit our video instruction pages. Note: since video and animation cannot be embedded in the print version of the journal, please provide text for both the electronic and the print version for the portions of the article that refer to this content.

Data visualization
Include interactive data visualizations in your publication and let your readers interact and engage more closely with your research. Follow the instructions here to find out about available data visualization options and how to include them with your article.

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

Research data

This journal encourages and enables you to share data that supports your research publication where appropriate, and enables you to interlink the data with your published articles. Research data refers to the results of observations or experimentation that validate research findings. To facilitate reproducibility and data reuse, this journal also encourages you to share your software, code, models, algorithms, protocols, methods and other useful materials related to the project.

Below are a number of ways in which you can associate data with your article or make a statement about the availability of your data when submitting your manuscript. If you are sharing data in one of these ways, you are encouraged to cite the data in your manuscript and reference list. Please refer to the "References" section for more information about data citation. For more information on depositing, sharing and using research data and other relevant research materials, visit the research data page.

Data linking
If you have made your research data available in a data repository, you can link your article directly to the dataset. Elsevier collaborates with a number of repositories to link articles on ScienceDirect with relevant repositories, giving readers access to underlying data that gives them a better understanding of the research described.

There are different ways to link your datasets to your article. When available, you can directly link your dataset to your article by providing the relevant information in the submission system. For more information, visit the database linking page.

For supported data repositories a repository banner will automatically appear next to your published article on ScienceDirect.

In addition, you can link to relevant data or entities through identifiers within the text of your manuscript, using the following format: Database: xxxx (e.g., TAIR: AT1G01020; CCDC: 734053; PDB: 1XFN).

Mendeley Data
This journal supports Mendeley Data, enabling you to deposit any research data (including raw and processed data, video, code, software, algorithms, protocols, and methods) associated with your manuscript in a free-to-use, open access repository. During the submission process, after uploading your manuscript, you will have the opportunity to upload your relevant datasets directly to Mendeley Data. The datasets will be listed and directly accessible to readers next to your published article online.

For more information, visit the Mendeley Data for journals page.

Data in Brief
You have the option of converting any or all parts of your supplementary or additional raw data into one or multiple data articles, a new kind of article that houses and describes your data. Data articles ensure that your data is actively reviewed, curated, formatted, indexed, given a DOI and publicly available to all upon publication. You are encouraged to submit your article for Data in Brief as an additional item directly alongside the revised version of your manuscript. If your research article is accepted, your data article will automatically be transferred over to Data in Brief where it will be editorially reviewed and published in the

open access data journal, Data in Brief. Please note an open access fee of 500 USD is payable for publication in Data in Brief. Full details can be found on the Data in Brief website. Please use this template to write your Data in Brief.

MethodsX
You have the option of converting relevant protocols and methods into one or multiple MethodsX articles, a new kind of article that describes the details of customized research methods. Many researchers spend a significant amount of time on developing methods to fit their specific needs or setting, but often without getting credit for this part of their work. MethodsX, an open access journal, now publishes this information in order to make it searchable, peer reviewed, citable and reproducible. Authors are encouraged to submit their MethodsX article as an additional item directly alongside the revised version of their manuscript. If your research article is accepted, your methods article will automatically be transferred over to MethodsX where it will be editorially reviewed. Please note an open access fee is payable for publication in MethodsX. Full details can be found on the MethodsX website. Please use this template to prepare your MethodsX article.

Data statement
To foster transparency, we encourage you to state the availability of your data in your submission. This may be a requirement of your funding body or institution. If your data is unavailable to access or unsuitable to post, you will have the opportunity to indicate why during the submission process, for example by stating that the research data is confidential. The statement will appear with your published article on ScienceDirect. For more information, visit the Data Statement page.

AFTER ACCEPTANCE

Online proof correction
Corresponding authors will receive an e-mail with a link to our online proofing system, allowing annotation and correction of proofs online. The environment is similar to MS Word: in addition to editing text, you can also comment on figures/tables and answer questions from the Copy Editor. Web-based proofing provides a faster and less error-prone process by allowing you to directly type your corrections, eliminating the potential introduction of errors. If preferred, you can still choose to annotate and upload your edits on the PDF version. All instructions for proofing will be given in the e-mail we send to authors, including alternative methods to the online version and PDF.

We will do everything possible to get your article published quickly and accurately. Please use this proof only for checking the typesetting, editing, completeness and correctness of the text, tables and figures. Significant changes to the article as accepted for publication will only be considered at this stage with permission from the Editor. It is important to ensure that all corrections are sent back to us in one communication. Please check carefully before replying, as inclusion of any subsequent corrections cannot be guaranteed. Proofreading is solely your responsibility.

Offprints
The corresponding author, at no cost, will be provided with a PDF file of the article via e-mail. The PDF file is a watermarked version of the published article and includes a cover sheet with the journal cover image and a disclaimer outlining the terms and conditions of use.

AUTHOR INQUIRIES

Visit the Elsevier Support Center to find the answers you need. Here you will find everything from Frequently Asked Questions to ways to get in touch.

You can also check the status of your submitted article or find out when your accepted article will be published