UNIVERSIDADE ESTADUAL DE MONTES CLAROS

João Marcus Oliveira Andrade

Avaliação dos Efeitos do Resveratrol sobre a Termogênese Adaptativa, a Ativação da Via da Irisina e o Processo de *Browning* no Tecido Adiposo de Camundongos e Humanos

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Tese apresentada ao Programa de Pós-graduação em Ciências em Saúde da Universidade Estadual de Montes Claros, como parte das exigências para a obtenção do título de Doutor em Ciências da Saúde.

Área de Concentração: Mecanismos e Aspectos Clínicos das Doenças

Orientador: Prof. Dr. Sérgio Henrique Sousa Santos

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RESUMO

Resveratrol é um polifenol que exerce benéficos efeitos sobre doenças associadas ao metabolismo corporal e ao envelhecimento/longevidade em diferentes espécies animais. A elucidação dos mecanismos moleculares modulados pelo resveratrol ainda permanecem desconhecidos. Assim, o presente estudo buscou avaliar os efeitos do tratamento oral com resveratrol sobre a ativação de marcadores envolvidos na termogênese, no processo de *browning* e na ativação do FNDC5/irisina no tecido adiposo de camundongos e humanos. Camundongos foram divididos em quatro grupos e alimentados por oito semanas com dieta normolipídica e hiperlipídica associadas ou não ao resveratrol. Durante o tratamento foram mensurados o peso corporal, consumo alimentar, consumo de oxigênio, nível de locomoção. Amostras de sangue foram utilizadas para dosagens de glicose, insulina, colesterol total, HDL, triglicerídeos, resistina, adiponectina e leptina. Análises da expressão de genes envolvidos nos processos de termogênese e *browning* e função mitocondrial foram realizadas por meio de qRT-PCR. Em seguida foram usadas amostras de tecido adiposo branco e marrom de vinte humanos obesos tratados ou não com resveratrol por quatro semanas. Além disso, foram realizados experimentos de cultura primária de adipócitos obtidos de tecidos humanos e animais. Os resultados foram agrupados em dois artigos. No primeiro artigo foi mostrado de o tratamento oral com resveratrol em camundongos tratados apenas com dieta normolipídica induziu uma significativa diminuição na adiposidade corporal, acompanhada por melhora nos níveis séricos de colesterol total e glicose de jejum. Além disso, pode-se observar aumento no consumo de oxigênio no grupo tratado com resveratrol. As análises moleculares por qRT-PCR revelaram aumento na expressão de genes envolvidos no processo de termogênese, como UCP1, SIRT1, PTEN e BMP-7 no tecido adiposo marrom. No segundo artigos, camundongos foram tratados com dieta normolipídica e hiperlipídica associadas ao resveratrol. Novamente, os resultados mostraram que o resveratrol induziu significativa melhora no perfil metabólico, diminuindo o peso e adiposidade corporal, melhorando a sensibilidade insulínica e tolerância à glucose, reduzindo os níveis de colesterol total e triglicerídeos. Esses resultados foram acompanhados por aumento nos níveis de expressão de marcadores associados ao processo de termogênese e *browning*, como UCP1, PGC1α e PRDM16 em diferentes tipos de tecido adiposo de camundongo e humano. Além disso, foi observado incremento da expressão de FNDC5/irisina no tecido adiposo subcutâneo visceral. O aumento desses marcadores parece ser parcialmente regulado pela SIRT1 como observado na cultura primária de adipócitos. Conclui-se que o resveratrol melhora a função metabólica de camundongos alimentados com dietas normo e hiperlipídica, induz o aumento na expressão de marcadores associados à termogênese e ao processo de *browning*. Além disso, induz a ativação do FNDC5/irisina parcialmente dependente de SIRT1.

Palavras-chave: termogênese, tecido adiposo, irisina, obesidade.

ABSTRACT

Resveratrol is a polyphenol that has beneficial effects on diseases associated to the body metabolism and aging/longevity in different animal species. The elucidation of the molecular mechanisms modulated by the resveratrol remains unclear. In this context, the present study aimed to evaluate the effects of the oral treatment with resveratrol on the activation of markers involved in the thermogenesis and browning processes and in the activation of the FNDC5/irisin pathway in the adipose tissue of mice and humans. The mice were divided into four groups and fed for eight weeks with standard and high-fat diet alone or associated with resveratrol. During the treatment, information such as body weight, food intake, oxygen consumption and locomotion level were analyzed. Blood samples were taken for the dosage of glucose, insulin, total cholesterol, HDL, triglycerides, resistin, adiponectin and leptin levels. Analysis of the expression of genes involved in the thermogenesis and browning processes and mitochondrial function were performed by qRT-PCR. Next, samples were taken from the white and brown adipose tissues of twenty obese human patients treated or not with resveratrol for four weeks. Furthermore, adipocytes obtained from human and mice tissue were used for primary culture experiments. The results were grouped in two papers. In the first paper it was shown that the oral treatment with resveratrol in mice fed with standard diet, induced a significant decrease of the body adiposity, accompanied by an improvement of the serum levels of total cholesterol and fasting glucose. Moreover, it was possible to observe an increase in the oxygen consumption in the group treated with resveratrol. The molecular analyzes by qRT-PCR showed an increase in the expression levels of the genes involved in thermogenesis, such as UCP1, SIRT1, PTEN and BMP-7 in the brown adipose tissue. In the second paper, mice were treated with standard and high-fat diet associated with resveratrol. Again, the results showed that the resveratrol induced a significant improvement of the metabolic profile, decreasing the body weight and body adiposity, improving the insulin sensitivity and glucose tolerance and reducing the levels of total cholesterol and triglycerides. These results were accompanied by an increase in the expression levels of the markers associated with thermogenesis and browning processes, such as UCP1, PGC1α and PRDM16 in different types of adipose tissues in humans and mice. Furthermore, it was observed an increment of the FNDC5/irisin expression in the subcutaneous and visceral adipose tissue. The increased expression of these markers seems to be at least partially regulated by the SIRT1 as observed in the primary culture of adipocytes. Therefore, it can be concluded that the resveratrol improves the metabolic function of mice fed with standard and high-fat diets, induces an increase in the expression of markers associated with the thermogenesis and browning processes and besides that, induces the activation of the FNDC5/irisin pathway partially dependent of SIRT1.

Keywords: thermogenesis, adipose tissue, irisin, obesity.

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1 INTRODUÇÃO

1.1. Obesidade

A obesidade consiste no acúmulo excessivo ou anormal de gordura corporal, resultante em um desiquilíbrio entre consumo e gasto energético (1, 2). É considerada umas das principais epidemias, com implicações individuais, sociais, econômicas e de saúde. A Organização Mundial da Saúde (OMS) estima para 2015 aproximadamente 2,3 bilhões de adultos com sobrepeso e mais de 700 milhões com obesidade no mundo (3). Para o Brasil, inquéritos populacionais conduzido na população adulta mostrou a prevalência de sobrepeso e obesidade em torno de 48,1% e 15%, respectivamente (4).

O aumento da prevalência de sobrepeso e obesidade afeta significativamente os indicadores de morbimortalidade e os gastos com serviços de saúde (5-7), além de ser importante fator de risco para doenças cardiovasculares (8-11), *diabetes melitus* tipo 2 (DMT2) (12-14) e câncer (15, 16), impactando negativamente na qualidade de vida e na longevidade da população (17-19). A estimativa total de custos associados às doenças relacionadas ao sobrepeso e à obesidade é de US$ 2,1 bilhões; US$ 1,4 bilhão (68,4% do total de custos) devido à hospitalizações e US$ 679 milhões devido a procedimentos ambulatoriais. Aproximadamente 10% desses custos são atribuídos apenas ao próprio sobrepeso e obesidade. Custos indiretos com a perda de anos de produtividade e qualidade de vida são inestimáveis (6).

A etiologia da obesidade é multifatorial, envolvendo fatores genéticos e ambientais (20, 21). Acredita-se que mudanças no padrão alimentar e no gasto energético estejam entre os principais fatores que contribuem para a etiologia da obesidade (22, 23). A mudança comportamental, no âmbito do padrão alimentar, inclui o aumento na ingestão de lipídios de origem animal, açúcares e baixo consumo de cereais e fibras (24-26). Paralelamente, a diminuição do gasto energético relacionada à inatividade física pode ser o mais importante fator que contribua para a obesidade (24, 27-29). Por outro lado, os aspectos moleculares envolvidos na obesidade e comorbidades associadas estão diretamente relacionados às propriedades biológicas de órgãos e tecidos metabólicos, como o tecido adiposo (TA) (30-32).

1.2 Tecido Adiposo

O tecido adiposo está cada vez mais envolvido nos processos homeostáticos e patológicos em mamíferos. Sua influência é exercida através de uma gama de funções e processos biológicos, a nível local e sistêmico. O TA é uma importante fonte de produção e liberação de citocinas (adipocinas) pró-inflamatórias e anti-inflamatórias, que têm um papel chave no desenvolvimento da obesidade e comorbidades relacionadas (33-35). Por outro lado, o TA pode também exercer benéficas funções corporais, por meio do processo de termogênese (36, 37).

O TA em mamíferos é composto por pelo menos dois tipos celulares funcionalmente distintos. O tecido adiposo branco (TAB) é um local primário para o armazenamento de energia e de liberação de hormônios e citocinas, que modulam o metabolismo corporal. Além disso, desenvolve atividades de isolamento térmico e proteção mecânica (32, 38, 39). O tecido adiposo marrom (TAM) é responsável pela produção química de energia usando fontes primária (lipídeos e carboidratos) para a produção de calor através da termogênese via fosforilação oxidativa de ácidos graxos livres mediada pela expressão de proteínas mitocondriais denominadas proteínas desacopladoras (UCPs) (37, 40). Como o TAB, o TAM pode afetar a homeostase corporal, sendo sua ativação umas das atuais estratégias para promoção da perda de peso e aumento da sensibilidade insulínica (41, 42).

Os adipócitos presentes do TAB possuem notável capacidade de alterar o fenótipo metabólico típico desse tecido para um com semelhantes características ao TAM (43-45). Esse fenômeno é referido como *"browning"* e é induzido por diversas condições como exposição ao frio, estimulação β-adrenérgica, exercício físico e uso de drogas/compostos naturais e é responsável pelo desenvolvimento de um novo tipo de tecido adiposo (Figura 1) (46, 47). O fenômeno de *browning* varia nos diferentes sítios de TAB e é fortemente influenciado por numerosos fatores genéticos (48-50). Tipicamente, o *browning* envolve o aparecimento de grupos de adipócitos multiloculares que estão dispersos no TA unilocular. Esses adipócitos foram chamados de "adipócitos bege" (*beige = brown + white*). Semelhante ao adipócito marrom, as células bege são definidas por usa morfologia constituída por gotículas de gordura multiloculares, com alto teor de mitocôndrias e pela expressão de um conjunto de genes próprios do TAM (UCP1, CIDEA, PGC1α e PRDM16) (43, 51). Apesar de sua capacidade de

induzir o processo termogênico, o tecido adiposo bege e marrom mantém muitas outras distintas funções, o que os torna tipos celulares distintos, entre elas o tipo da origem celular. Ao contrário dos adipócitos marrons, os beges são originados a partir de células progenitoras não miogênicas (não expressam Myf5$^-$) (52-54).

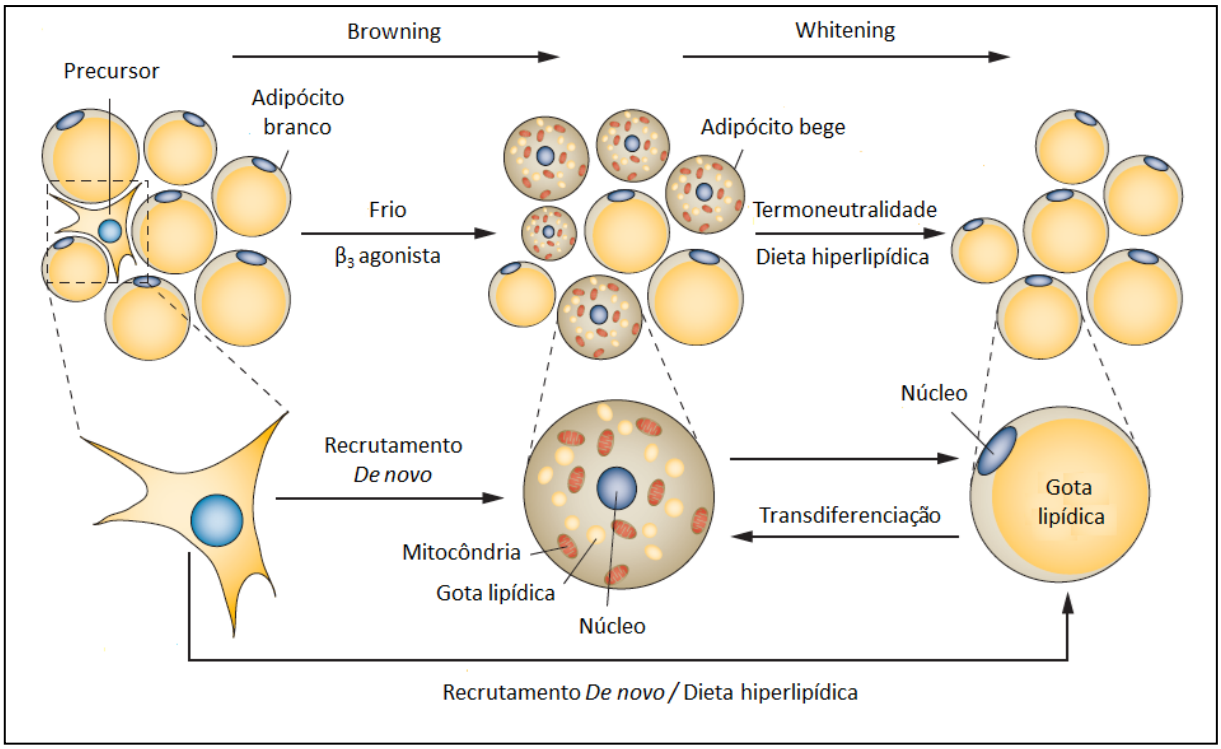

Figura 1 – Formação de adipócitos bege *(browning)* a partir do tecido adiposo subcutâneo e acúmulo de lipídeo em células morfologicamente remanescente dos adipócitos brancos *(whitening)*. Esses processos são dependentes do envolvimento de diversos fatores, como temperatura (frio) para o *browning* e dieta rica em gordura *(whitening)*. População de adipócitos precursores (Myf-5$^-$) podem diferenciar em adipócitos bege ou branco, dependendo da demanda ambiental (recrutamento *de novo*). No entanto, uma vez diferenciados essas células são morfologicamente flexíveis e podem adquirir fenótipos de adipócitos bege ou branco, respectivamente, quando submetido a estímulos de *browning* ou *whitening* (transdiferenciação). Adaptado de Bartelt e Heeren (2014) (54).

1.2.1 Tecido adiposo branco

O tecido adiposo branco (TAB) é um órgão que normalmente funciona como um reservatório corporal de energia. Em situações de excesso, acumula energia na forma de triacilglicerol (TG) e, em momentos de necessidade (jejum) libera catabólicos na forma de ácidos graxos (AG). O TAB mantém mecanismos de regulação homeostática através de mecanismos de regulação entre o armazenamento e a mobilização de gordura (31, 32, 55). Produtos oriundos

do catabolismo de AG possuem potenciais propriedade citotóxicas em órgãos periféricos. A capacidade do TAB em regular as variações entre oferta e demanda de energia é alcançada por mecanismos endócrinos e metabólicos, bem como, por alteração na composição celular. No entanto, em situações crônicas de excesso de oferta de nutrientes, ocorre desiquilíbrio desses mecanismos, resultando na liberação de gordura pelo TAB e, consequente acumulação patológica em outros órgãos e tecidos. Metabólicos derivados da acumulação de gordura ectópica prejudica diversas funções teciduais, como a produção, liberação e utilização de insulina, induz processos inflamatórios locais e lesão tecidual (56-60).

Durante décadas, o TAB foi considerado uma massa inerte de armazenamento de energia com algumas vantajosas propriedades, tal como o isolamento térmico e suporte mecânico para estruturas e órgãos importantes. No entanto, a partir da revolução no entendimento da função biológica desse tecido desde a última década, sobretudo com descoberta da leptina em 1994, proporcionou crescente interesse por esse órgão, agora essencial para a regulação da homeostase energética corporal, por meio do seu importante papel metabólico e biológico (30). Evidências indicam que o estado de inflamação crônica tem papel crucial na patogênese das disfunções metabólicas relacionadas à obesidade (34, 35). O TAB é um importante órgão endócrino responsável pela produção de citocinas (adipocinas) (35), que são hormônios protéicos tipicamente conhecidos como mediadores e reguladores de respostas imunes e inflamatórias (61, 62) (Figura 2). Em situações de obesidade, o estado secretório do TAB pode ser modificado por alterações na composição celular, incluindo no número e tamanho dos adipócitos, fenótipo e localização das células imunes, vasculares e estruturais (35). A expressão de adipocinas também pode variar dependendo da localização do TAB, sendo os dois principais e mais abundantes sítios, o visceral e o subcutâneo (63).

As adipocinas possuem papel importante na regulação da angiogênese, pressão arterial, homeostase da glicose, metabolismo lipídico e hemostasia vascular. Diversas adipocinas foram reconhecidas, mas em partes, mecanismos de atuação/regulação ainda permanecem pouco esclarecidos (64-66). No entanto, três delas exercem importantes funções locais e sistêmicas. A primeira adipocina com relevante destaque científico foi a leptina (67, 68). É um proteína altamente secretada pelo TAB. Atua por meio do receptor da leptina, o qual é expresso em vários órgãos e tecidos (69). A principal função da leptina é modular o consumo alimentar, por meio da regulação de hormônios, como o neuropeptídeo Y, corticoliberina e melanotropina-α (70, 71). A leptina também atua diretamente sobre o sistema nervoso central

(72, 73). A desregulação na produção e liberação da leptina pode induzir diversos processos maléficos, como potencialização do efeito aterogênico por indução da disfunção endotelial, estimulação da inflamação e geração de estresse oxidativo, aumento da agregação plaquetária e hipertrofia da musculatura vascular (74-76).

A resistina é uma adipocina diretamente envolvida na regulação da homeostase glicêmica, por meio da resistência insulínica (77-79). Níveis de resistina estão aumentados em indivíduos obesos e parece ser um dos principais fatores de relação entre obesidade e *diabetes mellitus* tipo 2 (79, 80). Além disso, resistina está associada à doenças cardiovasculares, sendo observado relação direta entre níveis de resistina e falência cardíaca e infarto do miocárdio (81-83).

Outra importante adipocina é chamada adiponectina. Ao contrário da leptina e resistina, níveis de adiponectina possui relação inversa com do índice da massa corporal (IMC) (84). A adiponectina atua por meio de dois receptores AdipoR1 e AdipoR2 (85, 86). Possui atividade anti-aterosclerótica e anti-inflamatória, aumento a sensibilidade insulínica, diminuição o processo inflamatório sobre o tecido adiposo e regula a homeostase lipídica em diversos tecidos (84, 87, 88).

Por fim, o TAB também é um exímio produtor de marcadores inflamatórios (Figura 2). Os adipócitos possuem propriedades inflamatórias intrínsecas. Eles expressam uma multiplicidade de receptores, sítios de ligação para diversas moléculas e células inflamatórias, que induzem a secreção de várias citocinas pró-inflamatórias e potentes mediadores químicos (60, 89). Adipócitos expressam receptores de fator de necrose tumoral alfa (TNF-α) (90), *toll-like receptor* 4 (TLR4) (91), que ao serem ligados ativam fatores transducionais, como fator nuclear kappa beta (NF-κβ) (92, 93), que inicia a liberação de uma cascata inflamatória sinalizada por diversas interleucinas (IL-1β, IL-4, IL-6, IL-11) (58, 94).

Os adipócitos não são as únicas células do tecido adiposo por eliciar respostas inflamatórias. Macrófagos residentes podem atuar de forma independente ou em sinergia estimulando o processo inflamatório (35, 95). Em indivíduos obesos, a população de macrófagos corresponde até a 60% do total de células presentes no tecido adiposo. Além disso, na obesidade induzida por dieta, macrófagos tendem a apresentar fenótipo proinflamatório (M1), envolvendo inúmeras citocinas, como IL-6, IL-12 e TNF-α (89, 96). O *status* de inflamação

local induzida por macrófagos pode se estender, como a alta produção de citocinas, induzindo processos inflamatórios sistêmicos, que contribuem para a fisiopatologia de diversas doenças.

Nesse contexto, o TAB é um tecido diretamente envolvido na função metabólica corporal, sendo a sua disfunção um dos principais fatores contribuintes para o desenvolvimento de inúmeros processos patológicos. O entendimento dos mecanismos envolvidos na regulação do tecido adiposo branco pode ser a chave para o desenvolvimento de abordagens terapêuticas para o tratamento da obesidade e doenças associadas.

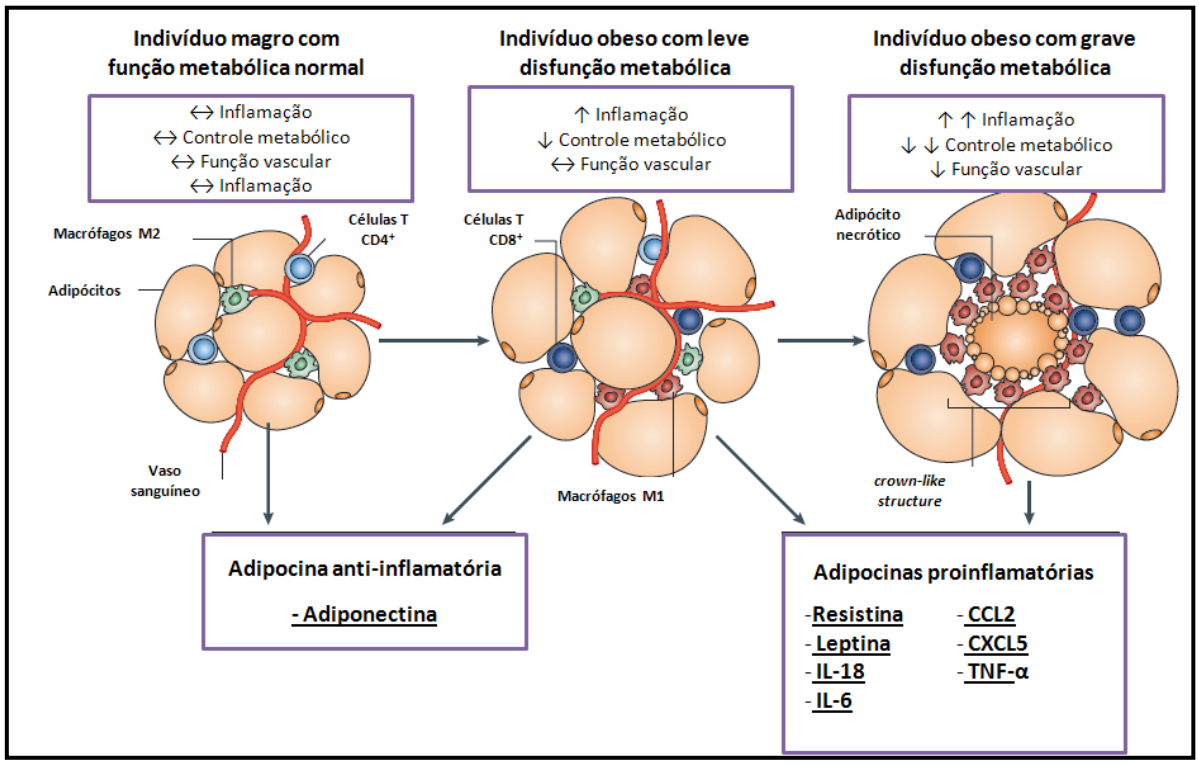

Figura 2 – Modulação fenotípica do tecido adiposo branco e produção de adipocinas. O tecido adiposo branco pode ser descrito estrutural e funcionalmente por três classificações fenotípicas: a) magro com função metabólica normal; b) obeso com leve/moderada disfunção metabólica; c) obeso com completa/grave disfunção metabólica. Como o estado de obesidade, os adipócitos sofrem hipertrofia, devido ao aumento no armazenamento de triacilglicerídeos. Com o estado de obesidade limitado, é provável que o tecido mantém a função metabólica relativamente normal e tem baixos níveis de ativação de células imunitárias e função vascular pouco alterada. No entanto, a expansão do tecido adiposo induz mudanças qualitativas, que induzem a transição para um fenótipo metabólico disfuncional. Estado de obesidade é acompanhado por aumento na população de macrófagos M1 e células T, com significativo aumento na produção de citocinas pro-inflamatórias, presença de necrose do tecido e formação de coroa de macrófagos. Adaptado de Ouchi *et al.* (35).

1.2.2 Tecido adiposo marrom

O TAM é formado durante o processo embriogênico antes de outros sítios de gordura BAT e contém uma população uniforme de adipócitos. O principal sítio de depósito do TAM em roedores está localizado na região interescapular (interescapular, axilar e cervical). Em humanos, inicialmente acreditava na presença de TAM apenas em neonatos, no entanto, estudos utilizando a técnica de PET-CT (Tomografia por Emissão de Pósitrons/Tomografia Computadorizada) constataram não apenas a existência do TAM, mas que em adultos ele continua a exercer seu papel primordial – a geração de calor (97-99).

A maioria das células de gordura marrom (interescapular) se originam a partir de células precursoras do mesoderma embrionário que também dão origem a células musculares esqueléticas e uma subpopulação de adipócitos brancos (100-102). Estes precursores transitoriamente expressam Myf5^{+}, gene que foi previamente pensado como marcadores seletivos para células miogênicas esqueléticas no mesoderma (102) (Figura 3).

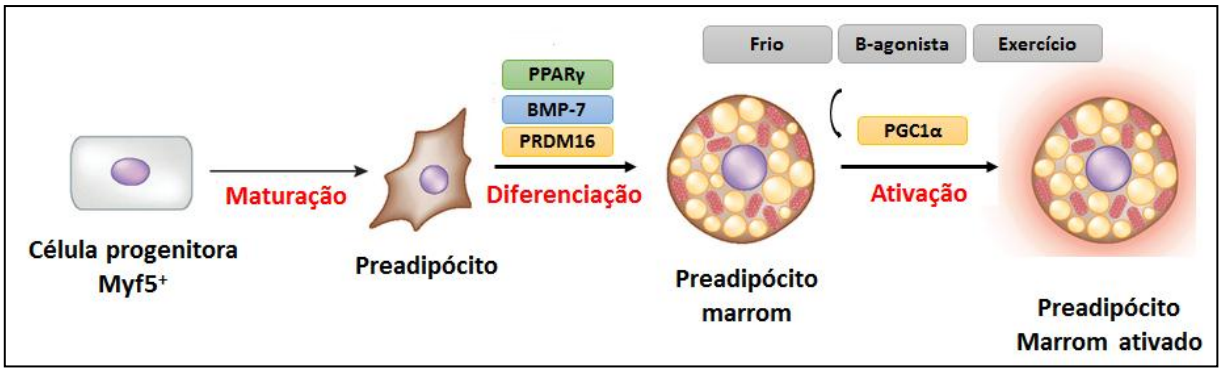

Figura 3 - Adipócitos marrons são derivados da população de células progenitores *Myf5*$^{+}$. Expressão de fatores como PPAR-γ, PRDM16 e BMP-7, induzem a diferenciação celular em preadipócito marrom. O processo de termogênese no tecido adiposo marrom é ativado por diferentes estímulos, como β3 agonistas, exercício físico e frio. Esses agentes induzem à ativação de PGC-1α, um coativador transcricional que coordena o programa gênico da termogênese no tecido adiposo marrom. Adaptado de Harms e Seale (103).

Os adipócitos marrons são multiloculares, pois contêm numerosas gotículas lipídicas contendo triacilglierol e, apresentam alta quantidade de mitocôndrias posicionas próximas à membrana plasmática. As mitocôndrias do TAM possuem numerosas cristas paralelas caracterizadas pela alta presença de proteínas desacopladoras (UCP) e pela pequena

quantidade de enzimas ATP sintetases (103-105). Essa característica morfológica e molecular constitui uma das explicações para a relação entre o TAM e o gasto energético corporal.

O TAM por ser rico em mitocôndrias, organelas envolvidas na regulação energética e por possuir alta expressão de UCP1 (proteína desacopladora 1). UCP1 é proteína localizada na membrana interna da mitocôndria, que tem como função a translocação dos prótons e elétrons do espaço intermembranas para a matriz mitocondrial, dissipando o gradiente de prótons através da membrana interna da mitocôndria (106, 107). Quando a UCP é estimulada, a energia não é aproveitada para a fosforilação do ADP, gerando apenas calor. A ativação desse caminho de translocação de prótons para a matriz mitocondrial resulta, indiretamente, em maior oxidação de substratos energéticos, diminuindo a eficiência da síntese de ATP e produzindo mais calor, com implicações na regulação da temperatura, do gasto energético e do peso corporal (105, 108). Portanto, o mecanismo de ação da UCP é desacoplar a fosforilação oxidativa da molécula de ADP (107, 109).

A UCP1 é uma proteína própria do TAM e confere a ele sua função termogênica (termogênese adaptativa independente de tremor), que consistiu-se como importante mecanismo regulatório da temperatura corporal por meio da produção de calor. O aumento da expressão de UCP1 determina consequentemente, o aumento na capacidade termogênica do TAM (105, 110).

Nos últimos anos, o TAM tornou-se alvo de inúmeros estudos, em virtude da elucidação de diversas funções desse órgão, que não apenas a produção de calor. Ao TAM foi atribuído importante papel na regulação do gasto energético corporal, perda de peso e consequentemente, melhora de parâmetros metabólicos, como a sensibilidade insulínica, fazendo do estudo desse tecido, importante estratégia para tratamento da obesidade e comorbidades associadas (111-116).

Importante estudo de Feldmann *et al.* (2009) revelou que camundongos deficientes para UCP1 apresentam maior ganho de peso do que camundongos *wild-type* (controle) em condições de termoneutralidade (105). Por outro lado, camundongos transgênicos com aumento na expressão de UCP1 (117) no tecido adiposo tornam-se resistes à obesidade induzida por dieta rica em gordura satura. UCP1 também tem sido expressa ectopicamente, como no músculo esquelético, induzindo a melhora na homeostase glicêmica em modelos

animais com obesidade induzida por dieta (117). Em humanos a expressão de UCP1 diminui significamente em indivíduos obesos no TAM. Nestes mesmos indivíduos também observa-se diminuição na expressão de UCP1 em sítios de TAB em relação a indivíduos magros (97, 118, 119). Por fim, estudo de Stanford *et al.* (2013) mostraram que o transplante de tecido adiposo marrom, induz aumento na expressão de UCP1 sistêmica e melhora a homeostase da glicose (41). Juntos, esses estudos indicam o importante papel da UCP1 local e ectópica e do tecido adiposo marrom na regulação da homeostase metabólica. A UCP1 é a principal molécula envolvida no processo de termogênese adaptativa (facultativa) independente de tremor. No entanto, outros fatores podem induzir o aumento da capacidade termogênica do TAM, ora por ativação da expressão de UCP1, ora por mecanismos independentes, mas ainda pouco conhecidos.

O TAM é um tecido com importante expressão do PGC1α (*Peroxisome proliferator-activated receptor-gamma coactivator*) é uma importante proteína envolvida na biogênese mitocondrial, regulação do estresse oxidativo e da termogênese (120-122). Elevação nos níveis de expressão de RNA mensageiro pode ocorrer no tecido adiposo marrom, coração, cérebro e rins (120). Exposição ao frio pode induzir significativo aumento na expressão PGC1α no TAB e músculo esquelético (123-125). PGC1α é um potente co-activador transcricional dos receptores de hormônios nucleares, incluindo membros da família PPAR. PGC1α parece coordenar a transcrição de genes que estão envolvidos na termogênese com a indução de UCP1, na cadeia respiratória mitocondrial, tais como ATP-sintetase e as subunidades (COX) II e IV do citocromo c oxidase, e na biogênese mitocondrial, por meio das ações sobre os fatores transcricionais NRF1, NRF2 e TFAM (126).

1.3 Termogênese

Em mamíferos, a termogênese adaptativa (TA) ocorre primariamente do tecido adiposo marrom e músculo esquelético. A TA pode ser dividida em três subtipos: a) Exposição ao frio induz a termogênese por tremor, uma função do músculo esquelético; b) termogênese independente de tremor, função do TAM; c) Termogênese induzida por dieta, também função do TAM (127).

A termogênese é essencial para animais de "sangue quente" (endotérmicos / homotérmicos), assegurando a manutenção da função celular e fisiológica normal em condições de estresse ambiental. Em humanos e roedores, durante a exposição prolongada ao frio, há o estado de cessamento dos tremores, acompanhada por preservação na taxa de gasto energético, devido à ativação da termogênese adaptativa independente de tremor (128). Em recém-nascidos, a manutenção da temperatura corporal é garantida devido à ativação do mecanismo de termogênese do TAM (129) .

Termogênese induzida por dieta foi descrita há mais de um século por Neumann (127). Consiste em um mecanismo fisiológico que permite que a ingestão excessiva de calorias seja dissipada na forma de calor (130, 131). Está associada à ativação do TAM por aumento na atividade adrenérgica (38) (132) e sofre interferência dos constituintes nutricionais, como carboidratos, proteínas e gorduras. Tanto a termogênese induzida por dieta quanto a independente de tremor compartilham de semelhantes características, como o mecanismos de regulação pelo sistema nervoso central e a produção de calor e gasto energético mediados pela UCP1 (133).

A termogênese é rigorosamente controlado por mecanismos nervosos e endócrinos, primariamente a nível de sistema nervoso central (SNC) (133). Em resposta ao frio, alimentação e β-agonistas o SNC regula em vários níveis o adipócito marrom. Esses estímulos induzem a proliferação e diferenciação de preadipócitos marrons, diretamente envolvidos na regulação do programa termogênico do TAM, sobretudo por meio de UCP1 (133, 134).

A termogênese adaptativa independente de tremor é o principal tipo de TA (Figura 4). Pode ser ativada nos adipócitos marrons por estimulação de receptores β-adrenérgicos (βARs), iniciando uma cascata de transdução de sinal que produz o AMP cíclico (AMPc) e ativa a proteína quinase A (PKA), a qual posteriormente ativa várias enzimas responsáveis pela conversão catabólicos de macronutrientes (carboidratos), gorduras (triacilgliceróis - TAG) e ácidos graxos livres (AGL)) e proteínas em combustíveis mitocondriais. O ciclo do ácido tricarboxílico (TCA) gera prótons (H^+) e elétrons (e^-) que são carregados por NADH e FADH para a cadeia de transporte de elétrons (ETC), onde os prótons são transportados para o espaço intermembranas (EIM), gerando um gradiente eletroquímico ($\Delta\mu H^+$) que é utilizado pelo F0F1-ATPase (ATPase) para converter o potencial energético em ATP (133, 135-137).

Enquanto isso, os elétrons são transportados em sucessivas etapas através dos complexos ETC [complexo I, NADH- ubiquinona (Q) oxidoredutase; complexo II, succinato – ubiquinona oxidoredutase; complexo III, ubiquinona - citocromo c (C) oxidoredutase e complexo IV, do citocromo -c oxidase] - até que sejam recebidos pelo O2 para produzir H2O. Os elétrons altamente reativos também levam à geração de espécies reativas de oxigênio (ROS), que pode causar danos celulares significativos. O TCA também produz CO2 como um subproduto. O quociente respiratório (RQ) é a relação de CO2 produzido ÷ O2 consumido tipicamente varia entre 0,7 e 1,0 para as gorduras de hidratos de carbono. Assim, RQ pode ajudar a identificar a fonte de combustível mitocondrial. UCP1 está localizada na membrana mitocondrial interna (MM) de adipócitos marrons, que permite prótons no espaço intermembranar da mitocôndria reentrem na matriz mitocondrial sem gerar ATP, isto é, não acoplado, e o calor é gerado neste processo (133, 135-137).

A termogênese adaptativa é também modulado por hormônios. Iodotironina desiodase tipo 2 possui papel vital na regulação entre a atividade do hormônio tireoidiano (triidotironina) e o TAM, modulando a termogênese adaptativa (138-140). Além disso, leptina liberado pelos adipócitos brancos, regula o equilíbrio de energia por efeitos sobre o hipotálamo, que levam à inibição da ingestão de alimentos e aumento da termogênese via ativação do SNC (141-147). Insulina pode afetar a termogênese, aumentando a absorção de substrato pelo TAM e a atividade simpática mediada pelo hipotálamo (148-150).

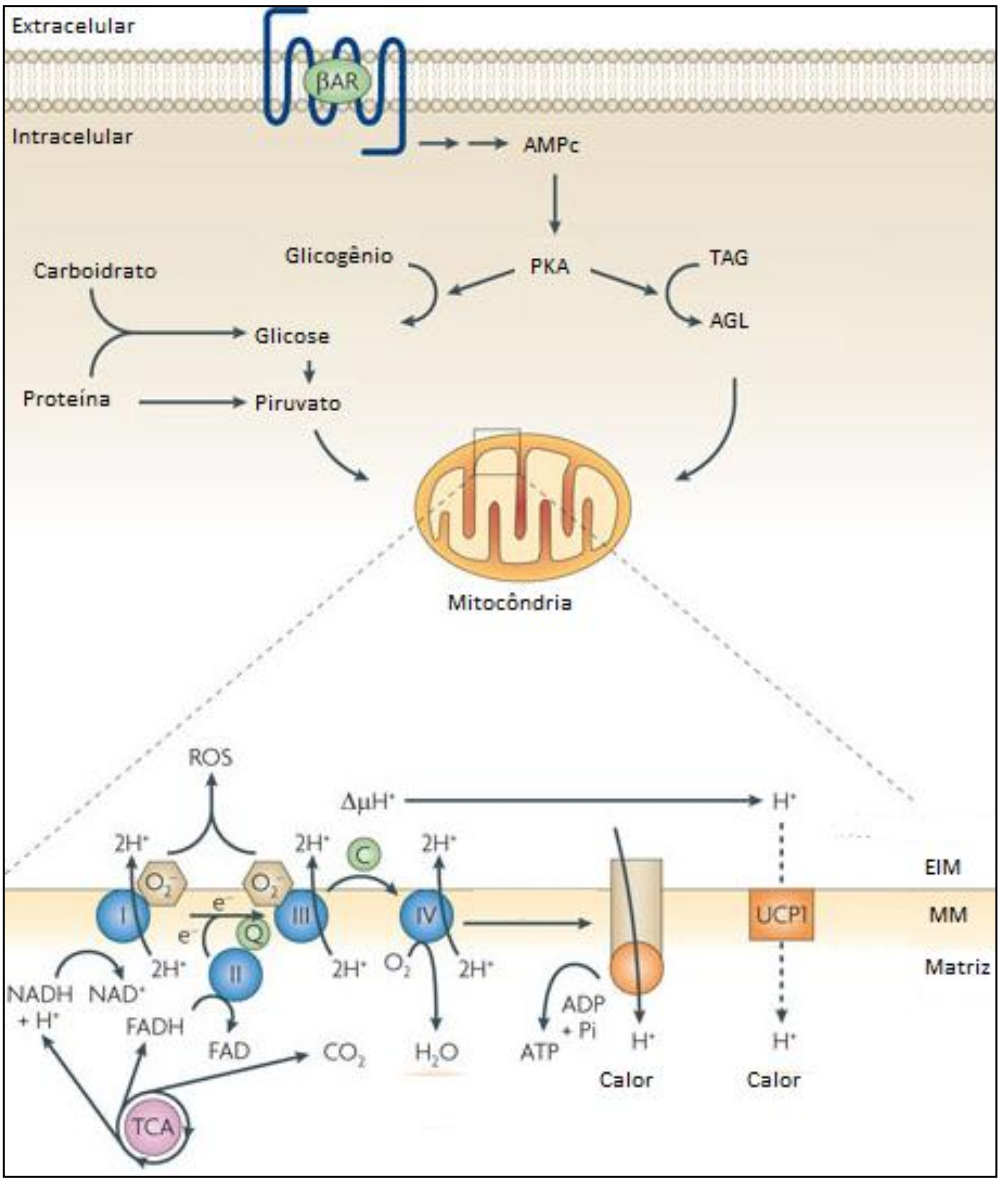

Figura 4 – Mecanismo de ativação da termogênese adaptativa independente de nos adipócitos marrons por estimulação de receptores β-adrenérgicos (βARs). Ativação de AMPc e PKA induz a conversão de catabólicos de macronutrientes em combustível usado no ciclo do ácido tricarboxílico (TCA), promovendo a geração de prótons (H+) e elétrons (e-), que serão usados pela cadeia transportadora de elétrons (ETC) para geração de energia na forma de ATP. O aumento na expressão de UCP1, induz a maior entrada de prótons do espaço intermembranar da mitocôndria para matriz mitocondrial sem gerar ATP, isto é, não acoplado, e o calor é gerado neste processo, induzindo assim, o gasto de energia. Adaptado de Tseng *et al. (149).*

1.4 FNDC5/Irisina

Há poucos anos, a existência do TAM em humanos era atribuída apenas neonatos, no entanto, novas evidências mostraram sítios específicos de TAM em humanos adultos metabolicamente

funcionais, distintas daquelas apresentadas pelo TAM presentes em neonatos (97-99). Posteriormente, dados sugeriram que os adipócitos marrons podem ser intercalados no TAB, formando uma população específica de adipócitos denominados, bege (43, 51, 52). Os adipócitos bege assemelham ao adipócitos brancos devido à baixa expressão de UCP1 em condições basais, mas podem responder a diversos estímulos, induzindo respostas metabólicas, como o aumento da expressão de UCP1 e aumento da capacidade de gasto energético, semelhantes aos observados no TAM interescapular (151).

O processo de *browning*, caracterizado pela diferenciação em adipócitos marrom no TAB é mais comum em sítios subcutâneos, especialmente o inguinal e tem se mostrado como umas das recentes estratégias para o tratamento da obesidade e melhora da homeostase glicêmica (152, 153).

Recente em artigo publicado na *Nature* por Boström *et al.* (122) teve intenso destaque midiático por conta de seu caráter inovador e promissor. Foi mostrado que o aumento nos níveis de PGC-1α nas células musculares decorrente da prática da atividade física induz a expressão de proteína de membrana tipo I, chamada FNDC5, a qual é clivada e secretada na circulação. A porção secretada na FNDC5 foi identificada como uma nova miocina conhecida como irisina, que liga-se a receptores indeterminadas na superfície dos adipócitos brancos. Por mecanismo parcialmente compreendido, a irisina induz a expressão de UCP-1 e outros genes associados no TAM, especialmente via aumento da expressão de PPAR- α. Assim, a irisina funciona como um sinal de gasto energético derivado do músculo que comunica diretamente com o TAB e induz o processo de conversão em TAM *(browning)* (122, 154-156). Esses efeitos melhoram o metabolismo tecidual e aumentam o gasto energético corporal total por incremento da termogênese adaptativa, fazendo a irisina um potencial alvo para o tratamento de doenças metabólicas, principalmente a obesidade (Figura 1).

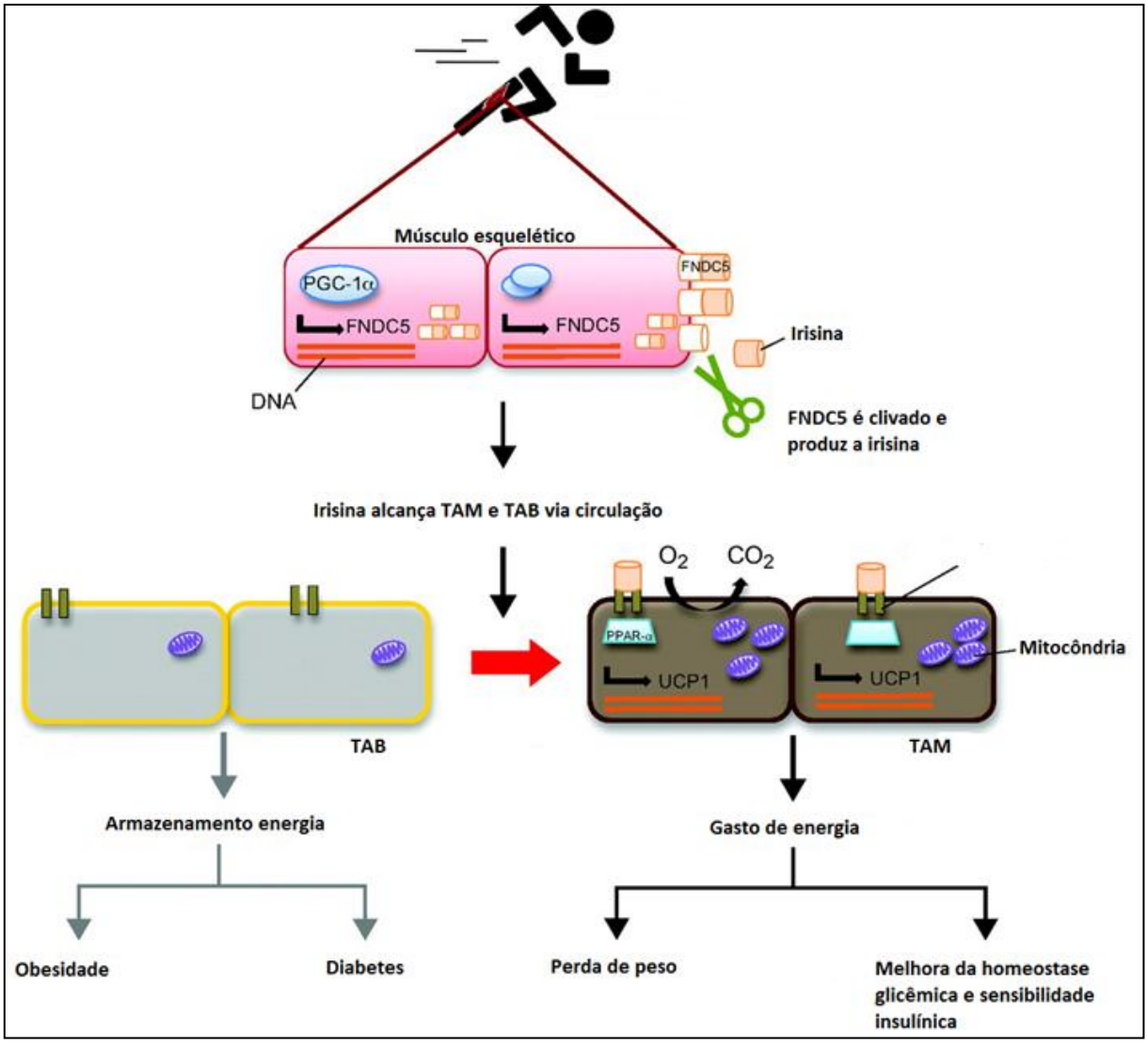

Figura 5 – Indução do *browning* do TAB através do PGC-1α e irisina. Exercício físico aumenta os níveis de expressão de PGC-1α no músculo esquelético. Isto, por sua vez, aumenta a expressão de FNDC5, uma proteína de membrana do tipo I, que é clivada na região C-terminal e liberada na corrente sanguínea como um novo hormônio – irisina. Irisina se liga a receptores desconhecido na superfície dos adipócitos do TAB, induzindo a expressão de PPAR-α, o qual atua como co-fator para o aumento da expressão de UCP-1 (proteína altamente expressa no TAB associada ao processo de *browning*). O *browning* está associado como o aumento da densidade mitocondrial e consumo de oxigênio, acompanhado pelo aumento do despêndio energético, contribuindo assim, para a perda de peso corporal e melhora em parâmetros metabólicos. Adaptado de Castillo-Quan (155).

Uma importante etapa neste contexto reside na identificação de vias e alvos moleculares específicos que subjazem o processo de *browning*. Boström *et al.* (122) identificaram o PPAR-α como fator chave para a expressão de UCP-1, mas há provavelmente outros genes envolvidos (122, 155).

Posteriormente a esse estudo, vários outros foram publicados identificando novas propriedades e mecanismos de ação do FNDC5/irisina. Hoje, sabe-se que além de uma

miocina, ela age como adipocinas, sendo produzida por diferentes sítios corporais de tecido adiposo branco (156). Níveis FNDC5/irisina foram diminuídos em pacientes com doença renal crônica e inversamente correlacionados com níveis sanguíneos de ureia e creatinina (157).

Em outro estudo, irisina estimulou o processo de *browning* do tecido adiposo branco através da ativação da via p38 MAPK e ERK, melhorando a homeostase metabólica por perda de peso e melhora na sensibilidade à insulina (158). No fígado, níveis de irisina estiveram significativamente diminuídos em pacientes obesos com doença hepática gordurosa não-alcóolica e esteato-hepatite não-alcóolica em relação a indivíduos saldáveis (159). No sistema nervoso, a elevação dos níveis séricos de irisina induziu o aumento na expressão do fator neuroprotetor derivado do cérebro (BDNF) e de outros genes neuroprotetores no hipocampo via PGC-1α/FNDC5 (160).

No entanto, em humanos o papel da irisina ainda permanece controverso. Estudo de Gouni-Berthold *et al*. (2013) mostrou que a expressão de mRNA de FNDC5 em biópsias humanas de músculo após exercício físico não apresentaram mudanças nos níveis de expressão (161). Além disso, preadipócitos humanos isolados do tecido adiposo subcutâneo manifestaram diferenciação para adipócitos bege sob incubação com BMP-7, mas não com FNDC5 recombinante, sugerindo que o papel do FNDC5/irisin em humanos parece comportar de forma distinta em relação a roedores (162).

Esses achados sugerem que apesar dos avanços recentes na compreensão dos mecanismos de ação do FNDC5/irisina sobre a homeostase metabólica em diversos órgão e tecidos, novos avanços são necessários, sobretudo na identificação de novos alvos e via de ativação da irisina.

1.5 Resveratrol

O resveratrol (3,4,5-tri-hidroxi-trans-estilbeno) é conhecido desde a década de 1940, quando foi primeiramente isolado a partir das raízes de heléboro branco e mais tarde a partir de *Polygonum cupsidatum*, uma planta medicinal (163, 164). O resveratrol está também presente

em quantidade apreciável em uma variedade de frutos comestíveis incluindo nozes, frutos vermelhos, e nas casca da uva (e derivados do vinho tinto). De um ponto de vista botânico, resveratrol atua como fitoalexinas, que é um composto tóxico produzido por uma planta em resposta a um ataque de parasitas, ou sob condições de stress (165).

A partir dos anos 80, intensificou-se os estudos destinados à investigação de compostos derivados de plantas dotados de propriedades farmacológicas (165). Extração de compostos bioativos a partir de *Polygonum cupsidatum* e *Poligonaceae* permitiram a identificação de um novo composto, o resveratrol (e outros estilbenos relacionados) como um inibidor de enzimas envolvidas no metabolismo o ácido araquidônico em leucócitos (166) e de quinases parcialmente purificadas (167). Embora estes dois estudos não terem avaliado o potencial terapêutico desses efeitos inibitórios em *in vivo*, eles estabelecem a base para uma investigação mais aprofundada sobre o resveratrol (165).

A busca de novos agentes quimiopreventivos ao câncer levou ao isolamento do resveratrol em outra espécie vegetal, a *Cassia quinquangulata*, baseado na inibição da ciclo-oxigenase 1 (COX-1) (168). O mesmo estudo demonstrou que a aplicação tópica de resveratrol diminuiu o edema de pata induzido por inflamação em ratos e retardou a progressão de câncer de pele (168), elevando assim o interesse no resveratrol como um composto anti-tumoral. Análise de possíveis alvos que induziram o aumento da expectativa de vida em *C. elegans* mostrou forte associação com o resveratrol, por meio de seu papel na ativação das sirtuínas (169). Como ativador das sirtuínas, a administração de resveratrol aumentou a expectativa de vida em leveduras em cerca de 70% (169), bem como em outras espécies como *C. elegans* e *D. melanogaster* (170), vertebrados inferiores (171) e camundongos (172). Em humanos, a suplementação diária de resveratrol induz significativa melhora em parâmetros metabólicos (173, 174).

Especulações sugeriram que a absorção de resveratrol por meio do consumo moderado de vinho tinto pode ser a explicação para o chamado “paradoxo francês”, que buscava uma resposta para o fato dos franceses, apesar da alta ingesta de dieta hiperlipídica, apresentavam baixa incidência de doenças cardiovasculares, quando comparados com outras populações da Europa (175). Essa evidência foi fortemente confirmada como estudos posteriores, que revelaram os efeitos cardioprotetores do resveratrol (176-179).

As ações do resveratrol podem ser explicadas pela sua vasta propriedade biológica de ativação e inibição de inúmeros alvos moleculares (180-182). O resveratrol exibe atividade anti-oxidante, anti-inflamatório, anti-tumoral, cardioprotetor e neuroprotetor, além de atuar positivamente na expectativa de vida de vários seres vivos. No entanto, recentemente, o resveratrol se tornou alvo de estudos mais aprofundados em virtude de sua capacidade de mimetizar os efeitos da restrição calórica (163). Tais efeitos biológicos são orquestrados por diversos mecanismos moleculares de ação do resveratrol no metabolismo celular, sobretudo por ativação das sirtuínas (183), que os fazem um potencial agente na prevenção e tratamento da síndrome metabólica e obesidade.

O resveratrol inibe a proliferação e diferenciação de preadipócitos (184), atenua a acumulação de lípidos no interior de células, como adipócitos, hepatócitos e células musculares e impede a liberação plasmática de ácidos graxos pelos adipócitos (185, 186), promove a diferenciação de células-tronco mesenquimais em osteoblastostos ao invés de adipócitos (187), aumenta o efeito lipolítico da adrenalina no tecido adiposo (188), estimula a absorção de glicose por células da musculatura esquelética (189), melhora a sensibilidade insulínica (190) e protege as ilhotas pancreáticas da citotoxicidade induzida por citocinas proinflamatórias (191). Além disso, camundongos alimentados com resveratrol minimizaram os efeitos da dieta rica em gorduras, melhorando a sensibilidade insulínica, hiperglicemia e dislipidemia (172). Em outro estudo, camundongos apresentaram significativa melhora no metabolismo após o recebimento de resveratrol associado à dieta hiperlipídica por 13 semanas, como melhora na sensilibidade à insulina, tolerância à glicose, aumento da taxa metabólica, incremento da resistência física e diminuição da gordura corporal (192). Embora, esse estudo não tenha avaliado a expressão de sirtuínas, o resveratrol alterou a atividade de diversas proteínas, algumas das quais são conhecidas por serem desacetiladas pelas sirtuínas (192).

Assim, as informações apresentadas acima indicam um promissor papel do resveratrol na regulação metabólica. No entanto, os alvos moleculares e as vias de sinalização e interação entre esses alvos permanecem desconhecidas.

1.5 Sirtuínas

A cromatina é formada por complexos de DNA associados a proteínas estruturais denominadas histonas e não histonas (193). Sabe-se que a cromatina exerce um papel importante na expressão gênica e que modificações como acetilação, desacetilação e fosforilação de histonas podem levar a mudanças na arquitetura dos nucleossomos e remodelamento da cromatina, o que pode resultar em alterações na transcrição gênica (194). As duas modificações mais estudadas são a acetilação e desacetilação de lisinas em histonas do núcleo, que são controladas por enzimas denominadas Histonas Acetiltransferases (HATs) e Histonas Desacetilases (HDACs) (193, 194).

Alguns padrões de expressão gênica das células resultam do balanço entre a atividade da HAT e da HDAC (195). Um descontrole na acetilação de histonas está relacionado com o desenvolvimento de diversas doenças como o câncer e alterações metabólicas (196-199). As HDACs são classificadas em 3 grupos principais: classes I, II e III (195, 200-202). A primeira caracteriza-se por proteínas com 350-400 aminoácidos (200), de expressão ubíqua e localizada quase exclusivamente no núcleo celular (201). A classe II é formada por HDACs de cerca de 1000 aminoácidos que movimentam-se entre o núcleo e o citoplasma em resposta à estímulos celulares (200, 201). As HDACs representantes da classe III são as sirtuínas (reguladores de silenciamento de informação – Sir2); filogenética e funcionalmente diferentes das outras duas classes devido ao seu mecanismo de ação único; dependente de nicotinamida adenina dinucleotídeo (NAD^+), enquanto as classes I e II são dependentes de zinco (201).

As sirtuínas fazem parte de um grande grupo de enzimas, a família das desacetilases de histonas ou HDACs, cuja função básica consiste na reversão da acetilação regulatória das proteínas tipo histona, o que influencia diretamente na estrutura dos nucleossomos e consequentemente na transcrição gênica (195, 203). A atividade desacetilase das sirtuínas é controlada pela razão celular $[NAD^+]/[NADH]$, onde NAD^+ atua como ativador e NADH e nicotinamida como inibidores (204). Além das histonas, um crescente número de proteínas tem sido identificado como alvo das HDACs, entre eles estão proteínas estruturais e diversos fatores de transcrição (202).

A partir da descoberta de parálogos de SIR2 em leveduras *Saccharomyces cerevisie*, logo foi

possível a identificação de homólogos em outros organismos. Já foi determinada a existência de genes ortólogos em todos os domínios de vida – bactérias, arquea, vírus e eucariontes (205) caracterizando SIR2 como membro de uma grande e antiga família de proteínas - as sirtuínas (206-208).

Nos anos 1990, Frye relatou a descoberta das primeiras cinco sirtuínas humanas (SIRT1-5) (209), e no ano seguinte identificou outras duas (SIRT 6 e 7) (209, 210). Durante suas pesquisas, identificou também que as sirtuínas de bactérias, leveduras e mamíferos seriam capazes de transferir grupos ADP-ribose do NAD^+ (209, 211), o que foi confirmado *in vitro* como uma função de ADP-risosil-transferase (211). Em 2000, Imai *et al.* (212) observaram que as histonas H3 e H4 poderiam aceitar o ADP-ribose do NAD^+ se estivessem acetiladas. Em seguida, foi descoberto que o principal mecanismo catalítico das proteínas SIR2 consiste na desacetilação de histonas e não a transferência de ADP-ribosil (211). A ação catalítica das SIR2 foi então co-caracterizada como desacetilase NAD^+-dependente por Landry *et al. (213)* e Smith *et al.* (214). A atividade desacetilase não foi identificada em uma sirtuína de mamíferos, a SIRT4, que exerce exclusivamente atividade de ADP-ribosil transferase dependente de NAD^+ (207, 215). A SIRT6 possui simultaneamente atividade desacetilase e ADP-ribosil transferase também dependente de NAD^+ (215). No entanto, ao contrário da desacetilação, a ação de ADP-ribosil-tranferase dependente de NAD^+ não é exclusiva das sirtuínas (216).

As sirtuínas estão conservadas não apenas entre eucariotos, mas também em bactérias e arquea (211). A partir de um estudo filogenético (210) foram divididas em cinco classes: I, II, III, IV e U. A última é exclusiva de sirtuínas bacterianas enquanto as classes I e IV são exclusivas de eucariontes (205). Mamíferos apresentam múltiplas sirtuínas, que diferem em função e localização celular (205, 208, 215).

As sirtuínas 1, 6 e 7 encontram-se predominantemente no núcleo e estão relacionadas com estabilidade do genoma e proliferação celular (217); SIRT2, presente no citoplasma, está envolvida no ciclo celular, e sua superexpressão prolonga a fase M (211). As SIRT3, 4 e 5 estão localizadas nas mitocôndrias e provavelmente estejam associadas ao metabolismo energético e a respostas relacionadas ao estresse oxidativo (217).
Originalmente, as sirtuínas demonstraram envolvimento apenas em silenciamento gênico. Porém, hoje se reconhece que estão envolvidas em diversas funções celulares muito além do

silenciamento transcricional (218, 219). Elas afetam uma ampla série de processos fisiológicos, incluindo regulação da expectativa de vida, regulação da atividade metabólica e enzimática, resposta celular ao stress, neurodegeneração, reparo de DNA, recombinação de DNAr, apoptose e controle de proliferação celular (205, 207, 208, 215, 218, 219). Atualmente, as sirtuínas são alvos importantes de pesquisas relacionadas à restrição calórica (208), câncer (220), doenças neurodegenerativas (217), diferenciação muscular, inflamação e obesidade (218).

As funções celulares das sirtuínas são distintas em cada proteína e em cada tecido ou órgão. Entre as sete sirtuínas, a SIRT1 é a mais estudada e, até o momento é a mais envolvida na regulação de vias metabólicas (221-223). A SIRT1 regula a expressão de adipocinas, como a adiponectina (138) e o fator de necrose tumoral (TNF-α) (224, 225), controla o balanço energético a nível hipotalâmico (226, 227), exerce importante papel da adipogênese e está envolvida na regulação da lipólise e na mobilização de ácidos graxos em resposta ao jejum (228, 229), atua na produção e sinalização da insulina em diversos tecidos alvos (190, 230) e regula positivamente a expectativa de vida (231).

Além disso, a SIRT1 exerce importante papel sobre a obesidade, síndrome metabólica e em comorbidades associadas (232-234). Evidências desse papel emergem da compreensão da função regulatória das sirtuínas em vias metabólicas relacionadas com à obesidade e à síndrome metabólica, como a expressão de citocinas pelos adipócitos (adipocinas), a maturação de células de gordura, secreção de insulina, regulação da síntese e liberação de ácidos graxos, modulação nos níveis plasmáticos de glicose, colesterol e triglicerídeos, controle da homeostase lipídica e regulação da função energética mitocondrial (222, 232, 234).

No músculo esquelético, SIRT1 induz a ativação de inúmeros marcadores que regulam a sua função. O aumento de SIRT1 induz o processo de deacetilação e ativação de PGC1α, aumentando a biogênese mitocondrial e da produção de enzimas envolvidas na oxidação de ácidos graxos, melhorando assim, a resistência muscular à atividade física, a força e a coordenação desse tecido (235). Outros estudos indicam, o papel da SIRT1 na ativação da biogênese mitocondrial no músculo esquelético por ativação de *AMP-activated protein kinase* (AMPK). Menzies *et al.* (236) demonstraram que o tratamento com resveratrol associado a exercício físico induz aumento da biogênese mitocondrial por efeito sinérgico dependente de

SIRT1, levando a translocação de PGC-1α para o núcleo e regulando assim fatores transcricionais da biogênese mitocondrial (236).

No tecido adipose marrom, SIRT1 ainda é muito pouco estudada. Lagouge *et al.* mostraram (237) que camundongos tratados com resveratrol associado à dieta rica em gordura apresentaram aumento do gasto energético e consume de oxigênio, com ativação de PGC1α dependente de SIRT1 (237). Recentemente, Alberti *et al.* mostraram que o resveratrol aumenta a expressão de UCP1 no tecido adipose marrom e músculo esquelético, acompanhados por aumento na expressão de SIRT1, também (238). Em outro estudo, Qiang *et al.* (239) mostraram que a deacetilação de PPARγ dependente de SIRT1 promove o processo de *browning* no tecido adipose subcutâneo, por regulação do complexo transcricional dependente de PPARγ. Esses resultados foram acompanhados por melhora da homeostase energética (239).

Em conjunto, esses achados mostram que o resveratrol/SIRT1 pode induzir termorregulação e melhorar a homeostase metabólica, sendo uma possível estratégia para tratamento da obesidade e doenças associadas,

2 OBJETIVOS

2.1 Objetivo geral

Avaliar os efeitos do tratamento oral com resveratrol sobre a ativação de marcadores envolvidos na termogênese adaptativa, no processo de *browning* e na ativação do FNDC5/irisina no tecido adiposo de camundongos e humanos.

2.2 Objetivos específicos

1. Avaliar os parâmetros antropométricos e metabólicos do resveratrol.
2. Avaliar a expressão de marcadores associados à termogênese adaptativa, à diferenciação de adipócitos marrons e à via de ativação do FNDC5/irisina no tecido adiposo de camundongos e humanos.
3. Avaliar os efeitos da ativação da SIRT1 sobre a expressão de FNDC5/irisina em cultura primária de adipócitos.

3 PRODUTOS

3.1 Artigo 1: "*Resveratrol Increases Brown Adipose Tissue Thermogenesis Markers by Increasing Sirt1, Energy Expenditure and Decreasing Fat Accumulation in Adipose Tissue of Mice fed a Standard Diet*", formatado segundo as normas para publicação do periódico European Journal of Nutrition (Fator de Impacto: 3,28; Qualis Interdisciplinar: A2). ***Status: publicado.***

3.2 Artigo 2: *"Resveratrol increase browning signalling-induced FNDC5/irisin up-regulating thermogenesis in adipose tissue of obese mice and humans",* formatado segundo as normas para publicação no periódico Diabetes (Fator de Impacto: 7,89; Qualis Interdisciplinar: A1). ***Status: submetido.***

3.1 Artigo 1

Resveratrol Increases Brown Adipose Tissue Thermogenesis Markers by Increasing Sirt1, Energy Expenditure and Decreasing Fat Accumulation in Adipose Tissue

João Marcus Oliveira Andrade*[3]; Alessandra Caroline Montes Frade*[1]; Juliana Bohnen Guimarães[2]; Kátia Michelle Freitas[1]; Miriam Teresa Paz Lopes[1]; André Luiz Sena Guimarães[3], Alfredo Maurício Batista de Paula[3], Cândido Celso Coimbra[2]; Sérgio Henrique Sousa Santos[1,3]

[1]Pharmacology Department, Biological Sciences Institute, Federal University of Minas Gerais (UFMG), Belo Horizonte, Minas Gerais, Brazil.
[2]Physiology and Biophysics Department, Biological Sciences Institute, UFMG, Belo Horizonte, Minas Gerais, Brazil.
[3] Laboratory of Health Science, Postgraduate Program in Health Sciences; State University of Montes Claros (UNIMONTES), Montes Claros, Minas Gerais, Brazil.

Short title: Resveratrol Increases Thermogenesis

* Equally contributed to this study
Correspondence may be addressed to:
Dr. Sérgio H S Santos
Departamento de Farmacologia
Universidade Federal de Minas Gerais
Av. Antônio Carlos 6627-ICB – 31270-901, Belo Horizonte, MG, Brasil
FAX/Phone: (55-31) 3409-2695/2724 - Email: sergiosousas@hotmail.com

Abstract

Purpose: Adipose tissue is central to the regulation of energy balance. Two functionally different fat pads are present in mammals: white adipose tissue, the primary site of triglyceride storage and brown adipose tissue, which is specialized in heat production. In this context, new strategies capable to modulate the development and function of white and brown adipose tissue become relevant. In the present study we analyzed the influence of resveratrol (Sirtuin activator) on energy balance and the expression of thermogenesis markers. **Methods:** Mice were divided into two groups: Standard diet (ST) and Standard diet plus resveratrol (ST+RSV). **Results:** After two months of treatment ST+RSV mice presented significant decrease fat accumulation in adipose tissue, with diminished total cholesterol and glucose plasma levels. Additionally, increased oxygen consumption was observed in ST+RSV group. Analyses of mRNA of thermogenesis-related genes showed significant increase in UCP1, SIRT1, PTEN and BMP-7 expression in brown adipose tissue. **Conclusion:** Our data suggest that improved metabolism produced by oral administration of resveratrol is, at least in part, associated with increased thermogenesis followed by high expression of UCP1 and SIRT1, which can mediate higher energy expenditure and decrease fat accumulation in adipose tissue.

Keywords: thermogenesis, PTEN, adipose tissue, SIRT1, UCP1.

INTRODUCTION

Adipose tissue is a key organ in the regulation of energy balance. Two functionally different types of fat are present in mammals: white adipose tissue, the primary site of triglyceride storage, and brown adipose tissue (BAT), which specializes in energy expenditure and thermogenesis (240). These two tissues differ in anatomical localization, abundance, maintenance throughout the life of the animal, morphology and mainly in function (241).

Adaptive thermogenesis (non-shivering thermogenesis), for the maintenance of basic body temperature and heat balance energy, is one important function of BAT. It is mediated by uncoupling proteins (UCPs), which are located in the inner mitochondrial membrane. These proteins act as uncouplers of oxidative phosphorylation by dissipating the proton gradient across the membrane and producing heat, rather than being used to drive the

synthesis of ATP (238). UCPs can, in this manner, dissipate surplus caloric energy and can consequently be important regulators of body weight.

In this context, thermogenic ingredients may be considered functional agents that could help to prevent a positive energy balance and obesity.

Resveratrol (3,5,4'-trihydroxystilbene) is a natural polyphenolic compound found in grapes and red wine, which has been shown to extend lifespan in many organisms (163, 172). It is a natural activator of the sirtuins (SIRT) family, the mammalian homolog of yeast silent information-regulator 2 (Sir2), and is comprised of a highly conserved family of proteins, with one or more sirtuins present in virtually all species from bacteria to mammals (221, 222). Previous studies have shown that resveratrol administration improves metabolic profile in mice and rats by modulating adipose tissue function (242, 243).

In a recent report it was shown that brown remodeling of white adipose tissue and thermogenesis are dependent on SIRT1 (239). Activation of NAD^+-dependent deacetylase SIRT1 by natural compound, calorie restriction or exercise, promotes mitochondrial biogenesis and activities (244, 245), raising the possibility that SIRT1 regulates BAT functions. Study published by Lagouge et al. showed that treatment of mice with resveratrol increased their aerobic capacity (increased running time and consumption of oxygen) by an induction of genes for oxidative phosphorylation and mitochondrial biogenesis, with increase in PGC-1a activity mediated by SIRT1 (237).

Alberti et al. showed that resveratrol increases the level of UCP protein expression in two importante thermogenic tissues (brown adipose tissue and skeletal muscle) suggesting that resveratrol can contribute to increased whole-body energy dissipation and consequently to increased energy expenditure, thus reducing energetic efficiency (238).

Taking these data into account, we hypothesize that oral administration of resveratrol in mice fed with a standard diet would modulate thermogenesis by increasing genes thermogenesis expression. This mechanism might contribute to alteration of energy efficiency, lipidic and glycemic profile and loss of fat mass.

MATERIALS AND METHODS

Animals and Diets

The experiment was conducted with sixteen male mice (four weeks old) from the Federal University of Minas Gerais (Belo Horizonte, Minas Gerais, Brazil) which were randomly divided into two groups (n=8 each) and fed the following respective experimental

diets for 8 weeks: Standard Diet (ST) and Standard Diet plus Resveratrol (ST+RSV) (4 g/kg of food) (237, 238).

Standard diet (Purina – Labina®), used for regular maintenance of the mice, is composed of 50.30% of carbohydrate, 41.90% of protein and 7.80% of fat with a total of 2.18 kcal per 1g of diet(246, 247).

All experimental procedures were approved by the Ethics Committee of the Universidade Federal de Minas Gerais for the Care and Use of Laboratory and were conducted in accordance with the regulations described in the Committee's Guiding Principles Manual.

Measurements of body weight, food intake, and tissue collection

Mice were individually housed, and food intake was measured twice a week during treatment to obtain food efficiency (food intake/body weight). After an overnight fast, mice were killed by decapitation. Samples of serum, epididymal, mesenteric and retroperitoneal white adipose tissue, interscapular brown adipose tissue and gastrocnemius muscle were collected, weighed, immediately frozen in dry ice and stored at -80ºC for subsequent analysis.

Oxygen consumption measurement

After 4 weeks of treatment, the animals were submitted to oxygen consumption measurement to verify thermogenic effects. Animals were transferred from their cages to an acrylic box where oxygen consumption (VO_2), an index of metabolic rate, was measured by an open-flow indirect calorimeter (PANLAB, Apparatus LE405, Gas Analyzer). The calorimeter was calibrated before each use with a certified mixture of gases (50% O_2 and 1% CO_2/ 20.2% O_2 and 0% CO_2–White Martins). Animals were allowed to rest at least 1 hour with VO_2 ($mLO_2 \times kg^{-1} \times min^{-1}$) being continuously recorded on-line using a computerized system (Metabolism OXYLET System). Data analyses used only the last 20 minutes of VO_2 recording, when animals had already returned to rest condition after the stress of handling. All experiments were conducted between 8:00 and 12:00 h to prevent circadian variation and ambient temperature was controlled at 23 ± 1°C (248, 249).

Locomotor activity

The experiments measuring the spontaneous locomotor activity (SLA) were carried out in an open field (40 cm in diameter with a 50-cm-high Plexiglas wall) located in an isolated room, and the lighting in the room was 200 lx. The animals were video-recorded, and the distance

moved was automatically analyzed with the aid of ANYMAZE software 4.5 (Stoelting, Wood Dale, IL, USA). For evaluating the effect of treatment on spontaneous locomotor activity, the animals were placed in the centre of the open field and the total distance moved in centimeters, was recorded during 30 min (250).

Determination of Circulating Biochemistry Parameters

Serum was obtained after centrifugation (3200 rpm for 10 minutes at 4°C). Total serum cholesterol, triglycerides, high-density protein (HDL) and glucose were assayed using enzymatic kits (Wiener®, Argentina). Measurements were made in Wiener BT-3000 plus Chemistry Analyzer (Wiener®, Argentina).

Quantitative Reverse Transcriptase Polymerase Chain Reaction – qRT-PCR

Total RNA from the brown adipose tissue was prepared using Trizol reagent (Invitrogen Corp.®, San Diego, California, USA), treated with DNAse and reverse transcribed with M-MLV (Invitrogen Corp.®) using random hexamer primers. Levels of UCP1, UCP3, Cidea, Adiponectin, PTEN, BMP-7, PRDM16 and SIRT1 mRNA were determined by qRT-PCR using SYBR Green reagent (AppliedBiosystems®, USA) in a PlusOne platform (AppliedBiosystems). Gene expression was normalized to the endogenous glyceraldehyde 3-phosphate dehydrogenase (GAPDH).

Statistical analysis

All data were transferred to GraphPad Prism software (Version 5.0®, San Diego, California, USA) and analyzed with 95% confidence ($P<0.05$). Data are expressed as the mean ± SEM. The statistical significance of differences in mean values among mice groups was assessed by T-student test.

RESULTS

ST+RSV mice did not present reduced final body weight ($P=0.72$) and food intake ($P=0.62$) (Fig. 1A and B). However, we observed significant decreases in epididymal (ST: 0.0075 ± 0.0051 vs. ST+RSV: 0.0022 ± 0.0019 g/body weight, $P<0.05$) and retroperitoneal (ST: 0.0012 ± 0.0006 vs. ST+RSV: 0.0006 ± 0.0003 g/body weight, $P<0.05$) adipose tissue in the group treated with resveratrol (Fig. 1C and 1E). No significant differences were found between ST and ST+RSV groups to mesenteric adipose tissue, skeletal muscle mass and

brown adipose tissue (Fig. 1E, 1F and 1G).

ST+RSV mice showed diminished total cholesterol (ST: 139.6 ± 9.39 vs. ST+RSV: 110.8 ± 11.52 mg/dL, *P<0.01*) and glucose levels (ST: 133.4 ± 22.19 vs. ST+RSV: 96.4 ± 15.13 mg/dL, *P<0.05*) (Fig. 2A and 2B). Regarding to HDL and triglycerides, no significant differences were observed between ST and ST+RSV groups (Figure 2C and 2D).

The oxygen consumption was increased in ST+RSV mice (ST: 36.59 ± 2.77 $mLO_2 \times Kg^{-1} \times min^{-1}$ vs. ST+RSV: 42.33 ± 1.35 $mLO_2 \times Kg^{-1} \times min^{-1}$; *P*<0.001) (Fig. 3A and 3B). Locomotor activity did not show difference between groups (ST: 104.9 ± 32.54 cm/30min. vs. ST+RSV: 80.32 ± 32.76 cm/30min.) (Fig. 3C).

Analyses of mRNA expression of thermogenesis-related genes showed significant increase in UCP1 (ST: 0.448± 0.15 vs. ST+RSV: 0.85 ± 0.22, *P<0.01*) (Fig. 4A). Other, targets like UCP3 (ST: 0.73 ± 0.10 vs. ST+RSV: 0.79 ± 0.24), Cidea (ST: 0.805 ± 0.183 vs. ST+RSV: 1.003 ± 0.492), PRDM16 (ST: 0.59 ± 0.13 vs. ST+RSV: 0.69 ± 0.28) and Adiponectin (ST: 0.933± 0.17 vs. ST+RSV: 0.895 ± 0.15) mRNA expression in the interscapular brown adipose tissue did not appear significantly different between groups (Fig.4B and 4E). Furthermore, an increase was observed in UCP1/UCP3 ratio in the same tissue (Fig. 4F).

Additionally, we observed a significant increase in SIRT1 (ST: 0.65± 0.19 vs. ST+RSV: 1.07 ± 0.19, *P<0.05*), PTEN (ST: 0.30± 0.12 vs. ST+RSV: 0.51 ± 0.08, *P<0.05*) and BMP-7 (ST: 0.32± 0.08 vs. ST+RSV: 0.51 ± 0.074, *P<0.05*) expression (Fig. 4G and 4I).

DISCUSSION

The main findings of this study are that mice treatment with resveratrol enhances thermogenesis and improves lipid and glycemic profile. These alterations were associated with increased UCP1 expression in brown adipose tissue. We suggest that high expression of SIRT1, PTEN and BMP-7 markers might be associated with increased expression of UCP1.

In the present study, the decreased body fat mass is not associated to alteration in total body weight. Previous studies have found that UCP1 levels are associated with changes in the adiposity index and significantly contribute to thermogenesis and adaptations of energy expenditure. Decrease in UCP1 expression is associated with increased adiposity (251, 252). Another study indicated that UCP1 enhances leptin action at the level of the hypothalamus, suggesting that this molecule contributes to the energy control balance not only through the regulation of energy expenditure but also through appetite control by modulating leptin action

(253).

Recent studies have shown that polymorphic variations of UCP1 gene has a strong association with reduced HDL-cholesterol levels, increased LDL-cholesterol levels (254, 255), or triglycerides (256), and increased systolic and/or diastolic blood pressure (257). In the other hand, UCP1 also appears to act in the regulation of glucose homeostasis. Several works have reported that polymorphisms -3826A/G, -1766A/G and -112A/C in the promoter region, Ala64Thr in exon 2 and Met299Leu in exon 5 of UCP1 gene are possibly associated with obesity and/or diabetes type 2 (258).

All these evidence suggest that increased expression of UCP1 is associated with a decrease in adiposity, is due to the increase in brown adipocytes numbers(259). Additionally, UCP1 induces an increase in oxygen consumption and energy expenditure, leading to weight loss. In fact, our study showed that resveratrol causes decrease in body adiposity, possibly by increasing UCP1 levels in brown adipose tissue.

Previous studies have found that aside from UCP1, UCP3 levels are also associated with changes in the adiposity index and significantly contribute to thermogenesis and adaptations of energy expenditure (250, 260). Our study revealed an increase in the ratio of UCP1/UCP3. Queiroz et al. (250) suggest that training increases the ratio between UCP1/UCP3 expression, which might mediate the induction of higher energy efficiency.

Mitochondrial biogenesis in brown adipose tissue and muscle is controlled, in large part, by the transcriptional coactivator PGC-1α (261), the activity of which, in turn, is positively regulated by SIRT1-mediated deacetylation (262). In our study, did not found increase in PGC-1α expression, but resveratrol induces increase in oxygen consumption. Moreover, Baur et al. suggest in your study that the acetylation status of PGC-1 α in the resveratrol-fed mice was threefold lower than the diet-matched controls. These findings were accompanied by increased mitochondrial biogenesis (172).

A recent report indicates that PTEN overexpression in mice presenting high levels of the UCP1 (263), accompanied by reduced body size, reduced levels of IGF1, improved insulin sensitivity, and increased energy expenditure. Moreover, transgenic PTEN mice have an increased energy expenditure, which is associated with lower adiposity and lower body weight despite being hyperphagic (263).

A new study showed that BMP-7 promotes the differentiation of brown pre-adipocytes (264). BMP-7 activates a full program of brown adipogenesis including induction of early regulators of brown fat rate PRDM16 (265) and PGC-1 (PPARγ coactivator-1) α (266), increased expression of brown fat defining marker UCP1 and adipogenic transcription factors

PPARγ, mitochondrial biogenesis via a p38 MAPK and PGC-1α dependent pathway (264). Additionally, Schulz et al. showed that genetic ablation of the type 1A BMP receptor (BMPr1a) in brown adipogenic progenitor cells leads to a severe paucity of BAT, affecting regulatory mechanism of control of the thermoregulation and energy homeostasis (152).

In addition, it was reported that a gain of function of NAD-dependent deacetylase SIRT1 promotes browning of WAT by deacetylating PPAR-γ on Lys268 and Lys 293 (239). SirT1-dependent deacetylation of Lys268 and Lys293 is required to recruit the BAT program coactivator PRDM16 to PPPA-γ, leading to selective induction of BAT genes and repression of visceral WAT genes associated with insulin resistance (239).

Lagouge et al. showed that mice treated with resveratrol increase energy expenditure and oxygen consumption, mediated PGC-1α activity through SIRT1 (237). Recently, other study Alberti et al. suggest that resveratrol increases in UCP1 protein expression in two important thermogenic tissues, brown adipose tissue and skeletal muscle (238). Together, these findings confirm the hypothesis new study showing that resveratrol via activation of SIRT1 can induce thermoregulation and improves metabolic homeostasis.

Taken together, our findings suggest that oral administration of resveratrol increases UCP1 expression by stimulation of SIRT1 which might mediate the induction of higher energy efficiency, improved metabolic parameters and decreased fat mass. The relationship between UCP1 and SIRT1 mRNAs might be responsible for the maintenance of body balance, allowing for changes in body composition, without change in body weight.

ACKNOWLEDGMENTS

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CONFLICT OF INTEREST

There is no conflict of interest to disclose for any of the authors.

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FIGURES

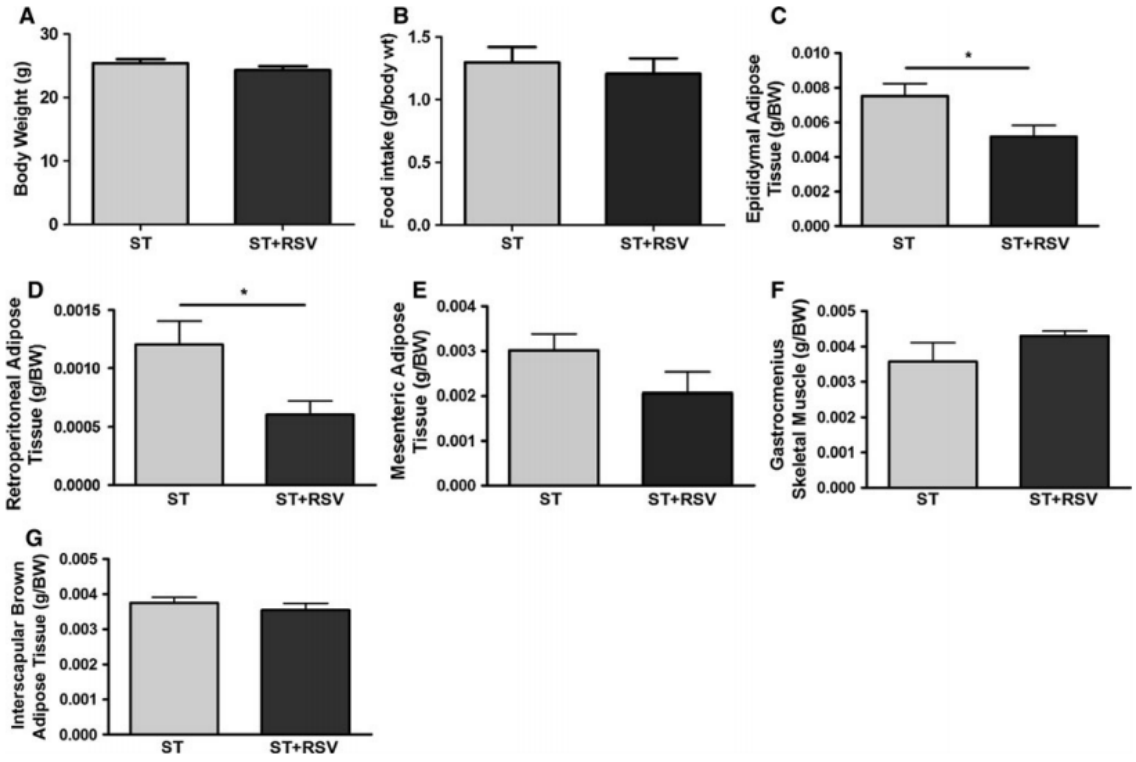

Fig. 1. Body weight, food intake and tissues weight. A: Body weight (g) of 4-week-old ST and ST+RSV (n=8) male mice. B: Food intake (g/g bw). C: Epididymal adipose tissue weight. D: Retroperitoneal adipose tissue weight. E: Mesenteric adipose tissue weight. F: Skeletal muscle weight. G: Brown adipose tissue weight. *P<0.05, in comparison to ST group.

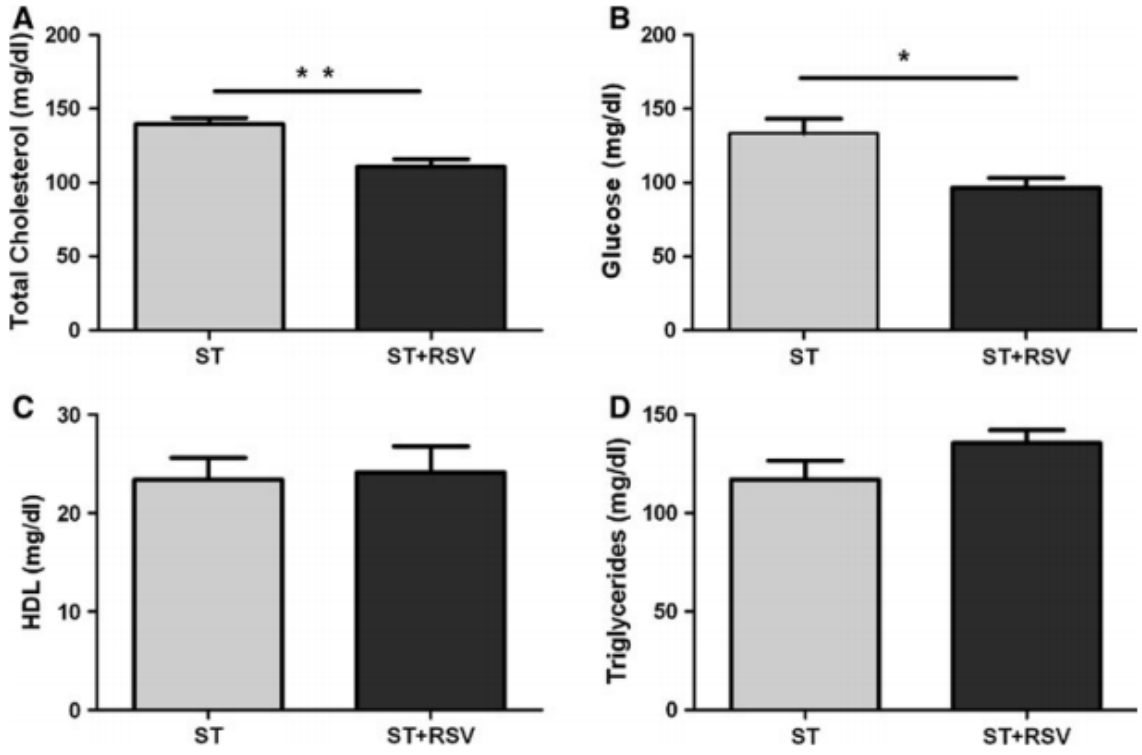

Fig. 2. Lipidic and glycemic profile. A: Plasma levels of total cholesterol. B: Plasma levels of glucose. C: Plasma levels of high-density protein. D: Plasma levels of triglycerides. *P<0.05 and **P<0.01 in comparison to ST group.

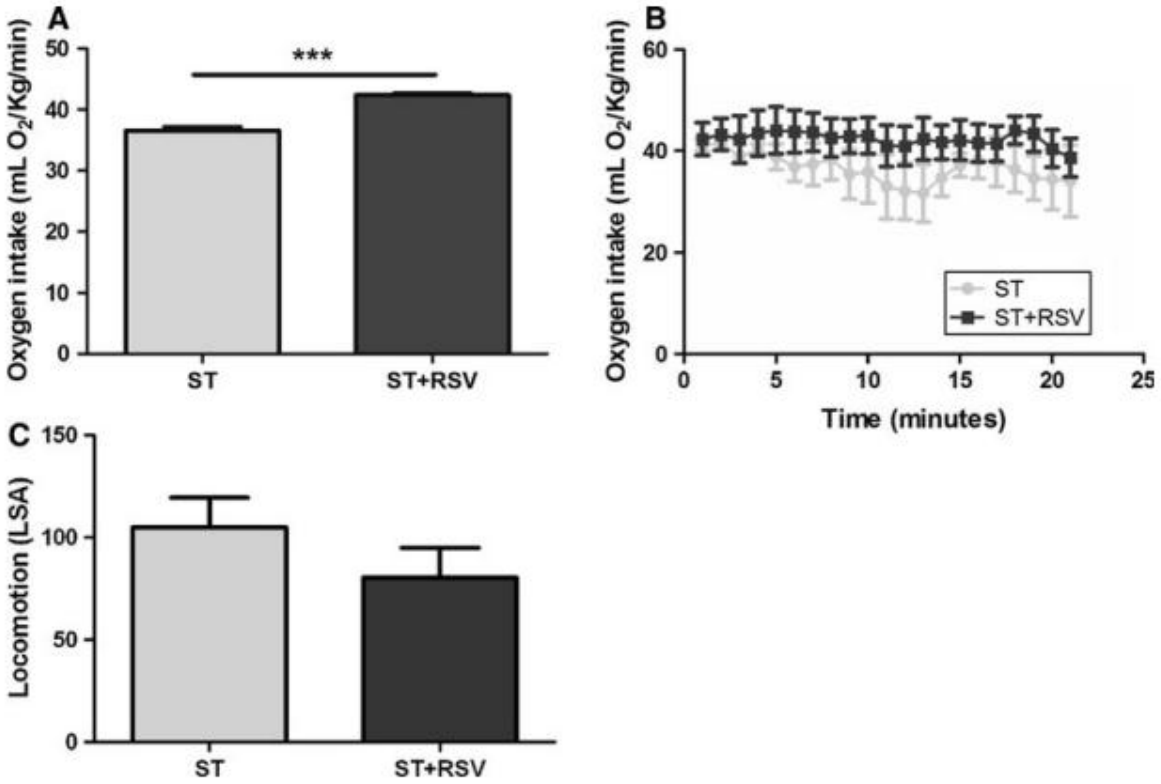

Fig. 3. Oxygen consumption and locomotion levels. A and B: Oxygen consumption (mL O_2/Kg/min). B: Locomotion levels (LSA). ***P<0.001 in comparison to ST group.

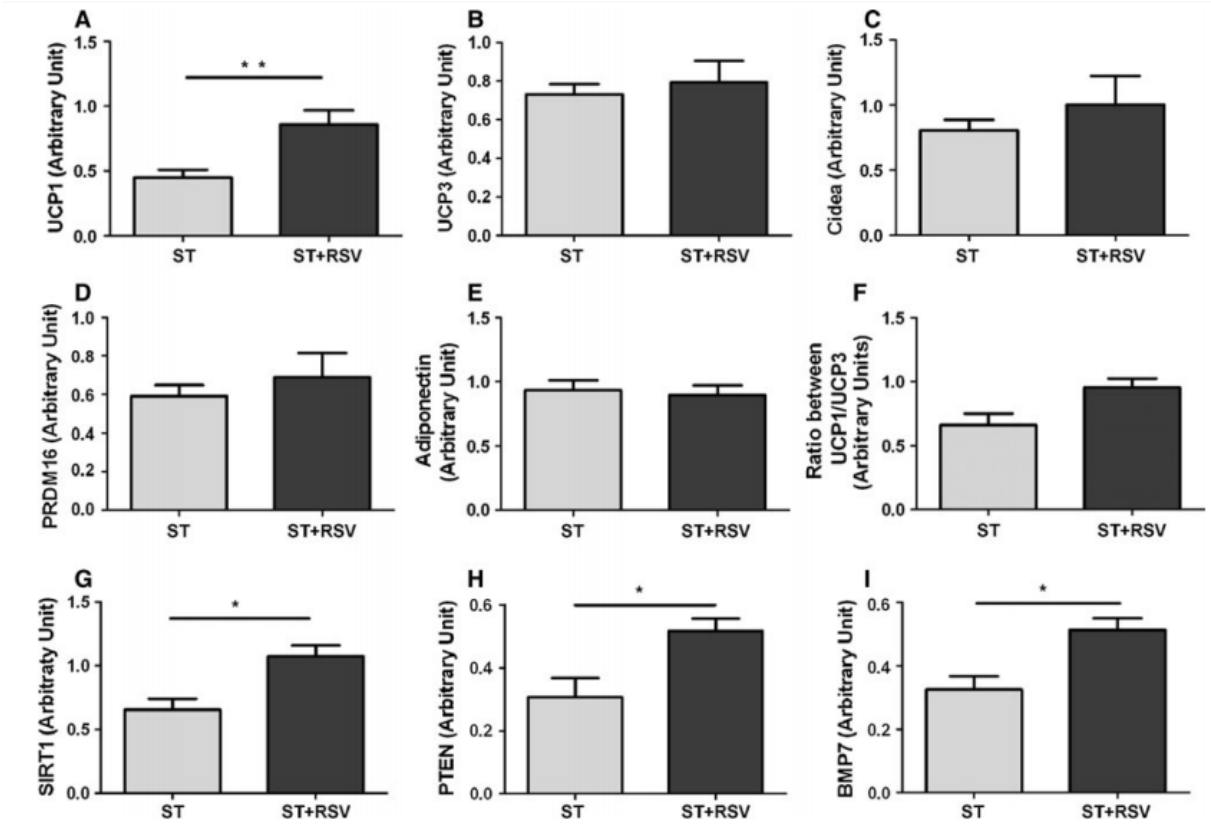

Fig. 4. Analyses of mRNA expression of thermogenesis-related targets by qRT-PCR in Brown-Adipose Tissue. A: mRNA expression of UCP1. B: mRNA expression of UCP3. C: mRNA expression of Cidea. D: mRNA expression of PRDM16. E: mRNA expression of adiponectin. F: mRNA expression of SIRT1. G: mRNA expression of PTEN. H: mRNA expression of BMP-7.*P<0.05 and **P<0.01 in comparison to ST group.

3.2 Artigo 2

Resveratrol increase browning signalling-induced FNDC5/irisin up-regulating thermogenesis in adipose tissue of obese mice and humans

João Marcus Oliveira Andrade[1] (MsD), Alanna Fernandes Paraíso[1](MsD), Kátia Michele de Freitas[2] (MsD); Deborah de Farias Lelis[1], André Luiz Sena Guimarães[1] (PhD), Alfredo Maurício Batista de Paula[1] (PhD), Sérgio Henrique Sousa Santos[1,2,*] (PhD)

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

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

Corresponding author:

Sérgio H S Santos

Laboratory of Health Science. State University of Montes Claros. Av. Cula Mangabeira 562. 31401-001, Montes Claros, MG, Brazil. FAX/Phone: (55-38) 3229-8327

Email: sergiosousas@hotmail.com

Abstract

Resveratrol provides clear beneficial effects for the prevention of several diseases. However, many of the molecular events responsible for the curative and protective role of resveratrol remain elusive. The recent discovery of FNDC5/irisin protein that is released by muscle and adipose tissue might be an important finding with regard to this unsolved mechanism. The

most striking aspect of this myokine/adipokine is its alleged capacity to drive brown-fat development of white fat and thermogenesis. Moreover, resveratrol might induces activation of key molecules involved in the regulation of thermogenesis and brown fat cell differentiation. Thus, our study is aimed to evaluate the resveratrol effects on FNDC5/irisin expression in adipose tissue in mice and human fed with high-fat diet. Our results showed that resveratrol induces significant ameliorate in metabolic profile such as decrease in body weight and body fat, improvement in insulin-sensitivity and glucose tolerance as well as lower plasma levels of fasting glucose and lipids. These results are followed by an increase in the levels of key molecules involved in the regulation of thermogenesis and brown fat cell differentiation like UCP1, PRDM16, PGC1α and SIRT1. In subcutaneous adipose tisseu, we observed an increase in the FNDC5/irisin expression only in mRNA levels. Nevertheless, we did not observe an increase in the FNDC5/irisin expression in brown adipose tissue. In conclusion, our findings provide evidence that resveratrol may be associated with activation FNDC5 and genes associated with browning and thermogenesis via SIRT1, especially in the mice a human subcutaneous adipose tissue. Finally, the present findings suggest new mechanism of action of the resveratrol.

Key words: resveratrol, adipose tissue, FNDC5, metabolism, thermogenesis, browning.

Introduction

In 2012, Boström *et al*. identified a new muscle-tissue-secreted peptide termed irisin (1). Interestingly, this myokine, whose secretion depends on the PGC-1α transcriptional co-activator activity, seems to be capable to induce white adipose tissue (WAT) browning (1). Increased levels of PGC-1α in muscle cells induced the expression of the type I membrane

protein FNDC5, which is cleaved and secreted into the circulation (1). The secreted portion of FNDC5, a newly identified myokine known as irisin, binds to undetermined receptors on the surface of WAT. By an incompletely understood mechanism, irisin induced the expression of UCP1 and other brown adipose tissue (BAT)-associated genes, partly via increased PPAR-α expression. Thus, irisin functions as a muscle-derived energy-expenditure signal that directly communicates with the adipose tissue and induces a brown-like genetic program, suggesting that a secreted molecule from the muscle cells is responsible for inducing browning of white adipose tissue (1-3).

Adipose tissues play major roles in the energy homeostasis and in the development of obesity and metabolic syndrome, which may be a new target against obesity and metabolic disorders (4). Adipose tissue insulin resistance and dysfunctional lipid storage in adipocytes are sentinel events in the progression toward metabolic dysregulation with obesity. Both subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT) are linked with metabolic risk factors. VAT remains more strongly associated with an adverse metabolic risk profile even after accounting for standard anthropometric indexes (5).

In this context, study using rat adipose tissue explants secretome proves that visceral adipose tissue, and especially subcutaneous adipose tissue, express and secrete FNDC5; hence, it may also behave as an adipokine. This effect improved the tissue metabolic profile and increased whole-body energy expenditure, making the irisin a potential new target for the treatment of metabolic diseases. Many other papers were published and indicate a benefic role of irisin in different organs and tissues (3). In this context, the discovery of new targets and activators of FNDC5/irisin emerges like promising alternatives for the prevention and treatment of obesity and metabolic disease.

Resveratrol (3,5,4’-trihydroxystilbene) is a natural polyphenolic compound found in grapes and red wine, which has been shown to extend lifespan in many organisms (6, 7).

Resveratrol is a natural activator of the sirtuin family, the mammalian homolog of yeast silent information-regulator 2 (Sir2), which is comprised of a highly conserved family of proteins with one or more sirtuins present in virtually all species from bacteria to mammals (8, 9).

Recently, studies indicated the effect of resveratrol on thermogenesis and browning process. Andrade *et al*. (10) indicate that resveratrol induced an improvement in body metabolism, like fat accumulation in adipose tissue, possibly by increasing of thermogenesis markers, like UCP1. Posteriorly, other study showed that resveratrol increases the level of UCP1 protein expression in two important thermogenic tissues (brown adipose tissue and skeletal muscle) suggesting that resveratrol can contribute to ameliorate whole-body energy dissipation and consequently to increase energy expenditure, thus reducing energetic efficiency (11).

Evidences indicate that brown remodeling of white adipose tissue and thermogenesis are dependent on SIRT1 (12). Activation of NAD^+-dependent, deacetylase SIRT1 by natural compounds, calorie restriction or exercises, promotes mitochondrial biogenesis and activities (13, 14), raising the possibility that SIRT1 regulates BAT functions. A study published by Lagouge *et al*. (15) showed that mice treated with resveratrol had their aerobic capacity increased (increase of the running time and consumption of oxygen) by an induction of genes for oxidative phosphorylation and mitochondrial biogenesis, with an increase in PGC-1α activity mediated by SIRT1 (15).

Given the relevance of the FNDC5/irisin discovery as well as its controversy and potential functions, it is necessary to obtain additional information regarding the nature of this hormone and its precise role in energy homeostasis. Resveratrol, a polyphenol with important functions in related-obesity diseases emerges as a promising possibility through its role in the thermogenesis and browning activation in the brown and white adipose tissue, respectively.

Therefore, our objective was to evaluate the effects of the resveratrol on the expression

of FNDC5/irisin in white adipose tissue of mice fed with high-fat diet and in humans, as well as its potential to induce the activation of key molecules involved in the regulation of thermogenesis and brown fat cell differentiation *in vivo* and *in vitro*.

2 Materials and methods

2.1 Drugs - Resveratrol was purchased from Sigma-Aldrich Co. LLC. (Saint Louis, MO, EUA). Daily dose (concentration of 0.04%) (6, 16).

2.2 Animals - The experiment was conducted with thirty-two male FVB/N mice (four weeks old) from the Universidade Estadual de Montes Claros (Montes Claros, Minas Gerais, Brazil). The mice were individually housed and placed in an air-conditioned room (22 ± 2°C) with a 12 h light-dark cycle. After an adaptation period of 7 days, they were randomly divided into four groups (n=8) and fed with experimental diets for 8 weeks: G1: Standard Diet (ST); G2: Standard Diet plus Resveratrol (ST+RSV); G3: High-fat Diet (HFD); G3: High-fat Diet plus Resveratrol (HFD+RSV). They had free access to food and water during the experimental period.

All experimental procedures were approved by the Ethics Committee from Universidade Estadual de Montes Claros for the Care and Use of Laboratory and were conducted in accordance with the regulations described in the Committee's Guiding Principles Manual.

2.3 Human Samples - Twenty male and female volunteers, aged 30-55 years, participated. All participants were obese (BMI >30 kg/m^2) but otherwise healthy, were taking no prescriptive medicine, and had no overt endocrine disorders. Eligibility ultimately was based on a normal physical examination including routine clinical biochemistry. The subjects were

divided in two groups and treated for 4 weeks with tablets containing 500 mg of *trans*-resveratrol (Fluxome Inc., Stenlose, Denmark) daily. During the trial period, the subjects were instructed to abstain from using nutritional supplements and consuming food suspected to contain resveratrol in significant amounts. Furthermore, the importance of maintaining their normal way of living was underscored.

The end of the treatment was performed under sterile conditions and using local anesthesia (Xylestesin® 2%, Cristália, Brazil) where adipose tissue biopsies (subcutaneous abdominal fat and interscapular brown adipose tissue) were obtained by liposuction, cleaned, and subsequently snap-frozen in liquid nitrogen.

2.4 Diets – High-fat diet was prepared according to the protocols described previously (17, 18), being composed by 24.55% carbohydrate, 14.47% protein and 60.98% fat, presenting a total of 5.28 kcal per 1g of diet. Standard diet (Purina - Labina®), which was used for the regular maintenance of our mice, is composed of 50.30% carbohydrate, 31.90% protein and 17.80% fat, presenting a total of 2.18 kcal per 1g of diet (19). All of the high-fat diet components were purchased from Rhoster® LTDA (São Paulo, Brazil).

2.5 Measurements of body weight, food intake and tissue collection - The mice were housed and the food intake was measured daily during the treatment to obtain food efficiency (food intake/body weight). Overnight fasted (12 hours) mice were killed after anesthesia with Ketalar® (130 mg/Kg - Pfizer Laboratório, Brazil) and Dorcipec® (0.30 mg/kg - Vallé S/A, Brazil) by decapitation. Samples of blood and white adipose tissue (epididymal, retroperitoneal, mesenteric and subcutaneous) and brown adipose tissue (intrescapular) were collected, weighed, immediately frozen in dry ice and stored at -80ºC for subsequent analysis.

2.6 Glucose tolerance and insulin sensitivity tests - For the glucose tolerance test, D-glucose (2 mg/g body weight) was intraperitoneally injected into overnight fasted mice. Glucose levels from tail blood samples were monitored at 0, 15, 30, 60, and 120 min after injection using an Accu-Check glucometer® (Roche Diagnostics, Indianapolis, USA). An insulin sensitivity test was performed on overnight-fed mice, after intraperitoneal injection of bovine insulin (0.75 units/kg body weight; Sigma-Aldrich®, St. Louis, USA). Tail blood samples were taken at 0, 15, 30, and 60 min after injection for measurement of blood glucose levels.

2.7 Determination of plasma parameters - Serum was obtained after centrifugation (600 x *g* for 10 minutes at 4°C). Total serum cholesterol, high-density lipoprotein (HDL) and triglycerides were assayed using enzymatic kits (Wiener®, Argentina). Enzyme-linked immunosorbent assay kits were used to measure serum adiponectin (Adipo-Gen®, Seoul, Korea), leptin (Abcam®, USA) and resistin (Lincoln®, St. Louis, USA) levels. Serum insulin was measured by chemiluminescence using a Rat/Mouse Insulin Kit (Millipore®, Billerica, USA) and ADVIA-Centaur equipment.

2.8 Reverse transcription and qRT-PCR - Total RNA from liver tissue was prepared using TRIzol reagent (Invitrogen Corp.®, San Diego, California, USA), treated with DNAse and reverse transcribed with M-MLV (Invitrogen Corp.®) using random hexamer primers. The endogenous glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (internal control), FNDC5, PGC1α, SIRT1, UCP1, PRDM16 cDNA samples were amplified using specific primers and SYBR green reagent (Applied Biosystems®, USA) in a PlusOne platform (Applied Biosystems®).

2.9 Primary Culture of Adipocytes - Adipocytes from FVB/N mice and human samples were isolated from white (subcutaneous and visceral) and brown (interscapular) adipose tissue and maintained in primary culture for 3 hours in DMEM containing 5 mmol/L glucose, 10% fetal bovine serum, 20 U/mL penicillin, 20 mg/mL streptomycin, and 1% BSA. The cells were incubated under basal conditions or in the presence of 50μM of Resveratrol, 10 μM of Sirtinol, or both (Resveratrol+Sirtinol). At the end of the incubation period, samples were collected and the mRNA expression levels of SIRT1, FNDC5, UCP1, FNDC5 and PGC1α were measured (20).

2.10 Statistical analysis - All data were transferred to GraphPad Prism software (Version 5.0®, San Diego, USA) and submitted to specific tests with a statistic confidence of 95% ($p<0.05$). Data were expressed as the mean ± SEM. The statistical significance of differences in mean values between mice groups were assessed by T-student test, one-way ANOVA or 2-way ANOVA (glucose tolerance and insulin sensitivity tests) and the Bonferroni post test.

RESULTS

In the first moment, we compared the effects of resveratrol in two different diets: standard and high-fat diet. The results indicated that the food intake per animal (g/BW/day) among all the groups was different only in ST group when compared with HFD group ($P<0.01$, Fig. 1A). However, energy intake (Kcal/BW/day) was significantly higher in HFD+RSV compared with ST and ST+RSV groups ($P<0.05$ and $P<0.01$, Fig. 1B).

Body weight (BW) was significantly lower in ST in comparison with HFD and HFD+RSV groups after eight weeks of high-fat diet feeding ($P<0.001$ and $P<0.01$, respectively, Fig. 1C). Concerning the body adiposity, it was observed a significant decrease in ST, ST+RSV and

HFD+RSV groups when compared to the HFD group (Figs. 1D and E) and increase in the brown adipose tissue of HFD+RSV when compared with HFD group ($P<0.05$, Fig. 1F).

Serum lipid measurements from HFD group revealed higher levels of total cholesterol (ST: 102.2 ± 16.35; ST+RSV: 103.5 ± 10.51; HFD: 173.2 ± 28.25; HFD+RSV: 115.8 ± 13.50, $P=0.003$); and triglycerides (ST: 140.3 ± 19.70; ST+RSV: 139.2 ± 18.97; HFD: 169.8 ± 13.78; HFD+RSV: 127.8 ± 17.59, $P=0.019$) than in the ST, ST+RSV and HFD+RSV groups (Figs. 2A and B). We did not find differences in the levels of high-density lipoproteins (HDL) among groups ($P=0.274$) (Fig. 2C).

Leptin levels were also increased in the serum of HFD group in comparison with HFD+RSV group (HFD: 23.42 ± 2.19 *vs.* HFD+RSV: 15.25 ± 2.013, $P=0.0022$) (Fig 2D). Additionally, serum adiponectin levels were increased between the groups HFD+RSV and HFD (HFD: 0.575 ± 0.0721 vs. HFD+RSV: 1.201 ± 0.142; $P=0.046$) (Fig. 2E). No difference was observed among groups regarding the resistin levels (Fig. 2F).

Posteriorly, intraperitoneal glucose tolerance test (IPGTT) and intraperitoneal insulin tolerance test (IPITT) were performed (Figs. 3A and B). The HFD+RSV group presented an important decrease of blood glucose levels at 15, 30 e 60 minutes after glucose administration when compared with the HFD group in both tests. The data were confirmed by the glucose area under curve test. This state was accompanied by an important increase in fasting plasma glucose and insulin levels in HFD group in comparison with all groups (Fig 3C and D).

The mRNA expression levels of targets involved in browning signalling and adaptative thermogenesis were analyzed in subcutaneous, visceral and brown adipose tissue of the experimental mice and human samples.

As shown in Fig. 4, the results revealed an increase in UCP1 ($P<0.001$), PGC1α ($P<0.01$), PRDM16 ($P<0.01$) and SIRT1 ($P<0.01$) expression in HFD+RSV group in comparison with

HFD (Figs. 4B and E) in mice brown adipose tissue. On the other hand, FNDC5 expression was not altered among groups (P=0.260) (Fig. 4A).

In mice subcutaneous adipose tissue, we observed an increase in UCP1 ($P<0.05$), PRDM16 ($P<0.05$) and SIRT1 ($P<0.01$) expression in HFD+RSV group in comparison with HFD (Figs. 5B, D and E). We did not find difference in the PGC1α expression levels among groups (P=0.538) (Fig. 5C). Moreover, the FNDC5 expression was decreased in the HFD group in comparison with ST and HFD+RSV groups, indicating an important role of resveratrol in FNDC5 expression (Fig. 5A). Posteriorly, analyses of visceral adipose tissue showed an increase in PRDM16 (P<0.01) and SIRT1 (P<0.05) expression in HFD+RSV group in comparison to HFD (Figs. 6D and E). Regarding the levels of FNDC5, UCP1 and PGC1α no differences were observed between the groups HFD+RSV and HFD (Figs. 6A and C).

In the second moment, we analyzed the browning signalling and adaptive thermogenesis markers expressions in brown and subcutaneous adipose tissue from obese volunteers treated for four weeks with tablets containing 500 mg of *trans*-resveratrol. The results indicated an increase in the levels of UCP1 (P<0.01), PRDM16 (P<0.05) and SIRT1 (P<0.05) in the brown adipose tissue of OBESE+RSV subjects in comparison with obese (Figs. 7B, C and E). Concerning the levels of FNDC5 and PGC1α, no difference was observed between groups (Figs. 7A and D). In subcutaneous adipose tissue we observed an important role of resveratrol in the activation of FNDC5 (P<0.01), UCP1 (P<0.01), PRDM16 (P<0.05) and SIRT1 (P<0.05) (Figs. 7F, G, H and I).

Finally, considering the described potential effects of resveratrol to stimulate browning and thermogenesis in adipose tissue and its potential effects on the modulation of FNDC5 via SIRT1, we evaluated the levels of the markers involved in brown fat cell differentiation in tissue and primary culture cell. The results indicated that resveratrol may be associated with

activation of FNDC5 and genes associated with browning and thermogenesis, especially in the mice and human subcutaneous adipose tissue (Figs. 8A and E).

4 Discussion

Recently, Boström *et al.* (1) published a promising mechanism for the induction of brite adipocytes in white adipose tissue depots after exercise in mice. Overexpression of PGC1α in mice skeletal muscle through exercise induced the expression of FNDC5. Posteriorly, FNDC5 is cleaved and the extracellular part of the protein is released, which acts as a novel molecule called irisin.

FNDC5 actions in mice caused browning of subcutaneous fat, stimulated oxygen consumption, and diminished diet-induced weight gain and metabolic dysfunction (1, 21). Thus, irisin induced a thermogenic mechanism in white adipose tissue, which improved whole body energy balance in mice (1, 2).

In this context, the discovery of the new activators of FNDC5/irisin emerges as an important preventive and therapeutic alternative to obesity-associated diseases. The involvement of resveratrol in the control of energy balance through the actions on food intake, body weight and energy expenditure has been previously reported in recent studies (6, 11, 15, 22, 23). Studies have implied that the resveratrol is an excellent protector against metabolic stress and obesity-related diseases in mammals. In part, the resveratrol's effects are due to the sirtuins activation (24-27).

For the first time, we show that resveratrol increase browning signalling-induced FNDC5/irisin up-regulating thermogenesis and its markers, such as UCP1, PRDM16 and PGC1α in adipose tissue of obese mice and humans, possibly via SIRT1.

White adipose tissue has an important role on energy homeostasis (18). Roca-Rivada *et al.* (3) showed that short-term periods of endurance exercise training induces FNDC5 secretion by white adipose tissue, suggesting that FNDC5/irisin acts like a new adipokine, which has autocrine function in adipose tissue (3). However, the molecular mechanisms and cellular signaling pathways responsible for the effect of irisin have not been elucidated.

Many studies were published in the last year about FNDC5/irisin mechanisms in different organs and tissues (2, 3, 21, 28-31). In our study, we showed that resveratrol should be an important activator of FNDC5/irisin. Several signaling pathways and key molecules activated by resveratrol such as PGC1α, MAPK, SIRT1 and UCP1 are important in FNDC5 activation and thermogenesis induction.

The sirtuin/Sir2 is a family of NAD1-dependent deacetylases and mono-ADP-ribosyltransferases proteins. In mammals, seven sirtuin genes have been identified (SIRT1–7) (32-34). SIRT1 regulates processes such as glucose and insulin production, fat metabolism, and cell survival, leading to the speculation that sirtuins might mediate effects of caloric restriction in mammals. It has been shown that a significantly increase in SIRT1 activity through an allosteric interaction, results in the increase of Sirt1 affinity for both NAD^+ and acetylated substrate (27).

In our study, we showed that resveratrol might shift the physiology of mice and human on a high-calorie diet through the modulation of key molecules involved in the regulation of thermogenesis and brown fat cell differentiation. Activation of the NAD^+-dependent deacetylase SIRT1 by small molecules, calorie restriction or exercise promotes mitochondrial biogenesis and activities (13, 16, 25), raising the possibility that SIRT1 regulates white/brown adipose tissue functions.

Qiang *et al.* (12) showed that SIRT1-dependent PPARγ deacetylation promotes browning of subcutaneous WAT by regulating ligand-dependent coactivator/ corepressor

exchange at the PPARγ transcriptional complex. Furthermore, it was identified that SIRT1-dependent PPARγ deacetylation regulates energy homeostasis, promoting energy expenditure over energy storage (12).

Recently, Alberti *et al.* (11) suggested that resveratrol increases the UCP1 protein expression in two important thermogenic tissues, brown adipose tissue and skeletal muscle (11). Additionally, in other study, Lagouge *et al.* (15) showed that mice treated with resveratrol had an increase in the energy expenditure and oxygen consumption, an improvement in the mitochondrial function and protection against metabolic disease mediated by PGC-1α activity through SIRT1 (15). Together, these findings confirm the hypothesis that resveratrol induces activation of SIRT1, which may increase thermoregulation and improves metabolic homeostasis. Andrade *et al*. concluded that improved metabolism produced by oral administration of resveratrol is, at least in part, associated with increased thermogenesis followed by high expression of UCP1 and SIRT1, which can mediate higher energy expenditure and decreased fat accumulation in adipose tissue.

Browning signaling is an important mechanism involved in increase energy expenditure, preventing weight gain and consequently, may be considered a fundamental strategy for the prevention of diet-induced obesity and increased insulin sensitivity. Browning of rodent WAT can be mediated by hormones and cytokines, such as irisin (1), SIRT1 (12, 15), Fgf21 (35), Wnt (36) and PRDM16 (37). In our study, we investigated if resveratrol might confer BAT-like features onto WAT.

PRDM16 is a large zinc finger–containing transcriptional factor that is highly expressed in mouse and human BAT when compared to its expression in visceral and adjacent subcutaneous WAT (38, 39). Studies suggested that PRDM16 acts primarily through binding to and modulating the activity of other transcriptional factors, including PGC1α (40-42).

Seale et al. demonstrated in two studies that the down-expression of PRDM16 induces ablation of the thermogenic characteristics of brown fat cells while causing an increase in the expression of white fat–specific and muscle-specific genes (38, 40). Together these studies have strongly suggested that PRDM16 is a key driver of brown fat cell fate.

Various stimuli induce thermogenic capacity by UCP1 up-expression in white adipocytes (43, 44). These adipocytes have been named beige, brite (brown in white). Similar to adipocytes in BAT, beige cells in mouse WAT are defined by their multilocular lipid droplet morphology, high mitochondrial content and the expression of a core set of brown fat–specific genes. Despite a common ability to undergo thermogenesis, brown and beige cells have many distinguishing characteristics and should be considered as distinct cell types. Beige adipocytes are more abundant in the subcutaneous WAT. In special situations, as cold exposure, UCP1-expressing adipocytes are evident in all WAT depots (45-47).

Adaptive thermogenesis is defined as heat production in response to the environmental temperature, diet, drugs, hormones and cytokines and is mediated by uncoupling proteins (UCPs), which are located in the inner mitochondrial membrane. UCP1 is an integral membrane protein unique to brown adipocyte mitochondria, where it acts as a proton channel to uncouple oxidative phosphorylation by dissipating the proton gradient across the inner mitochondrial membrane (44).

In our study, treatment with resveratrol induced an increase in UCP1 expression in BAT and subcutaneous WAT. Alberti et al. (11) found similar data, showing that resveratrol induced a significant increase in SIRT1 expression in BAT and in PGC1α, which is a potent inducer of mitochondrial biogenesis, an important part of the thermogenic program. Increase in PGC-1α expression activates the UCP1 gene. In addition, Langouge et al. demonstrated that in the BAT and WAT, RSV treatment induced striking mitochondrial morphological

changes and also increased UCP1 expression levels and thus poised the mitochondria for uncoupling of respiration (15), inducing browning in white adipose tissue.

In conclusion, the results obtained in the present study showed that resveratrol induces significant ameliorate in metabolic profile. Additionally, it was observed an increase in the browning signalling-induced FNDC5/irisin up-regulating thermogenesis and its markers, such as UCP1, PRDM16 and PGC1α in adipose tissue of obese mice and humans possibly via SIRT1. All together, these findings may help us to better understand the body-fat lowering effect of resveratrol. However, mechanisms that involve the possible role of resveratrol in the activation FNDC5/irisin pathway brown/white adipose tissue need to be better understood.

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Disclosure statement

The authors declare that they have no competing interests.

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Figures legends

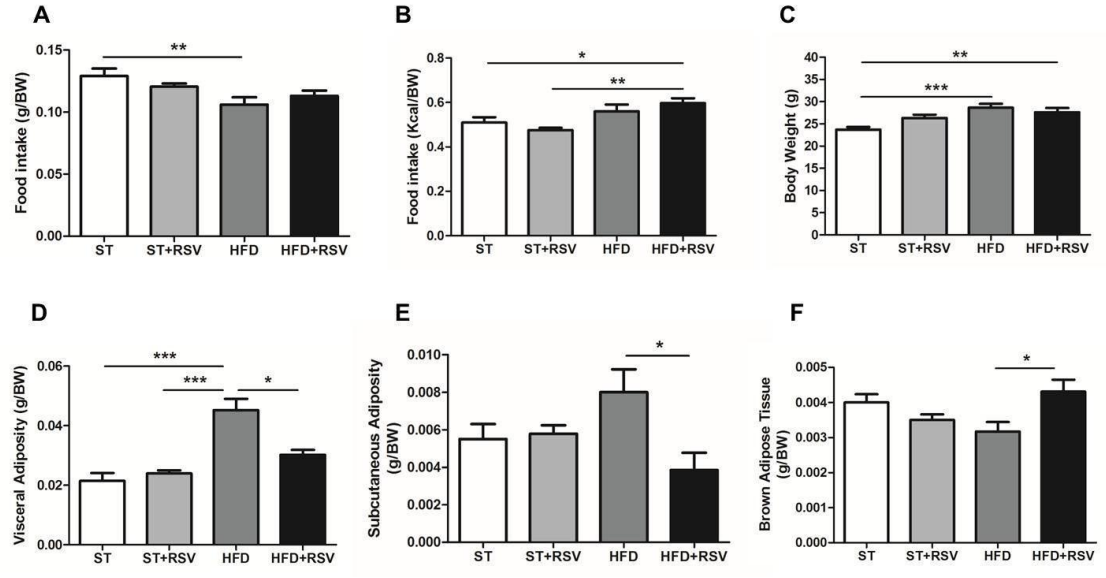

Figure 1. RSV Prevents Diet-Induced Obesity. A) Food intake (g/BW). B) Energy intake (Kcal/BW). C) Body Weight (g). D) Visceral Adiposity (g/BW). E) Subcutaneous Adiposity (g/BW). F) Brown Adipose Tissue (g/BW). Differences between groups were analyzed by one-way ANOVA followed by Bonferroni's *post hoc* test. Data are presented as means ±SEM. **P*<0.05, ***P*<0.01 and ****P*<0.001.

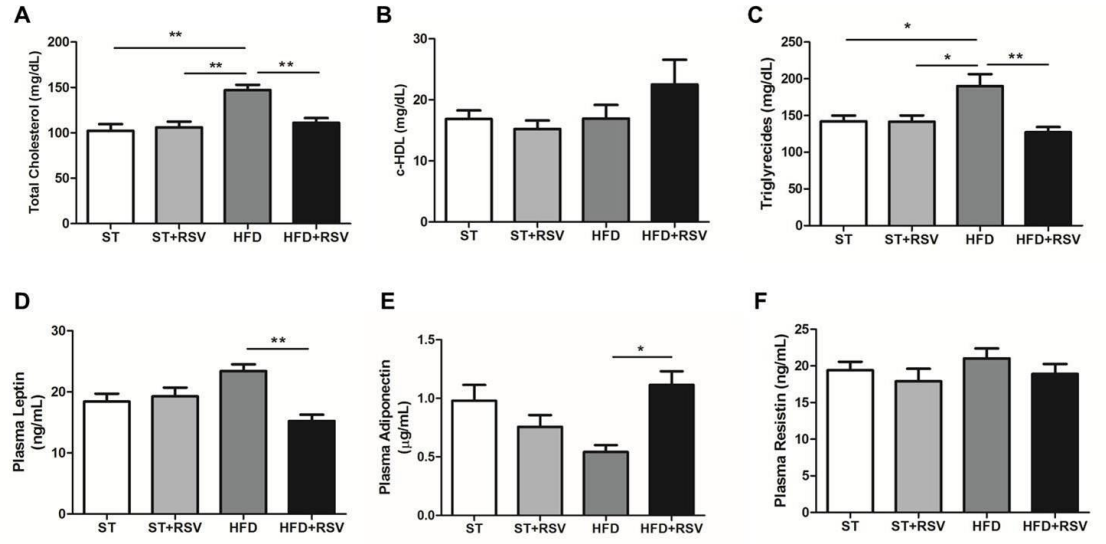

Figure 2. Resveratrol ameliorated lipidic plasmatic profile. A) Total cholesterol (mg/dL). B) c-HDL(mg/dL). C) Triglycerides (mg/dL). D) Plasma levels of leptin (ng/mL). E) Plasma levels of adiponectin (µg/mL). F) Plasma levels resistin (ng/mL). Differences between groups were analyzed by one-way ANOVA followed by Bonferroni's *post hoc* test. Data are presented as means ±SEM. **P*<0.05, ***P*<0.01 and ****P*<0.001.

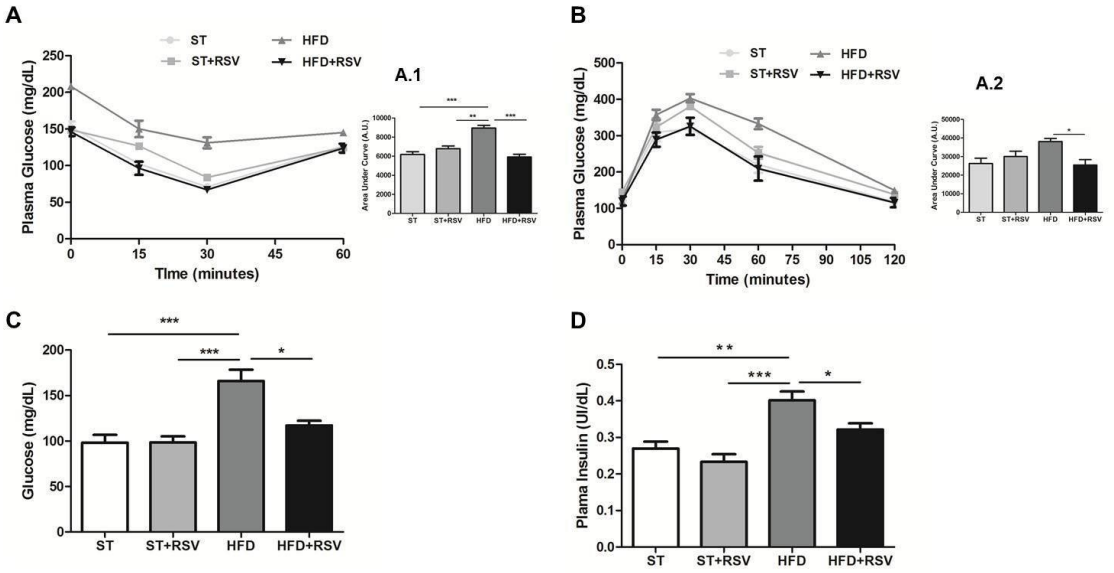

Figure 3. Resveratrol ameliorated glycemic plasmatic profile. A) Intraperitoneal Insulin Tolerance Test (IPITT) and IPITT glucose area under the curve. B) Intraperitoneal Glucose Tolerance Test (IPGTT) and IPGTT glucose area under the curve. C) Plasma glucose (mg/dL). D) Plasma insulin (UI/L). Differences between groups were analyzed by one-way ANOVA followed by Bonferroni's *post hoc* test. Two-way ANOVA (glucose tolerance and insulin sensitivity tests) and the Bonferroni *post hoc* test. Data are presented as means ±SEM. **P*<0.05, ***P*<0.01 and ****P*<0.001.

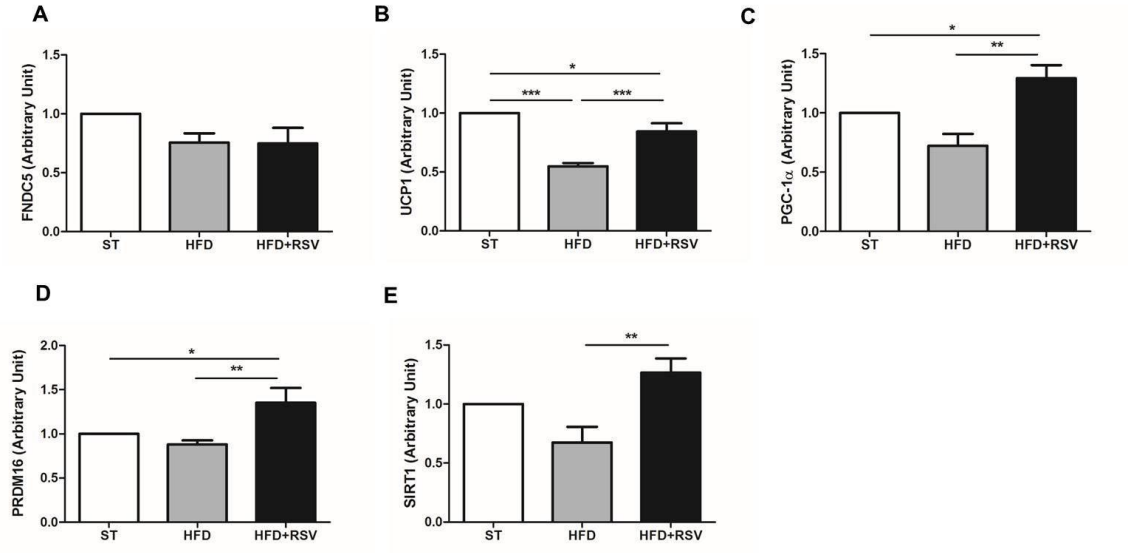

Figure 4. Resveratrol increases expression of FNDC5, SIRT1 and thermogenesis markers in mouse brown adipose tissue. A) FNDC5 mRNA expression. B) UCP1 mRNA expression. C) PGC-1α mRNA expression. D) PRDM16 mRNA expression. E) SIRT1 mRNA expression. Gene expression data were normalized for the expression of GAPDH and gene expression levels in ST group were assumed to be 1. Values are the mean ± SEM (n= 6 per group). Differences between groups were analyzed by one-way ANOVA and the Bonferroni *post hoc* test. Data are presented as means ±SEM. **P*<0.05, ***P*<0.01 and ****P*<0.001.

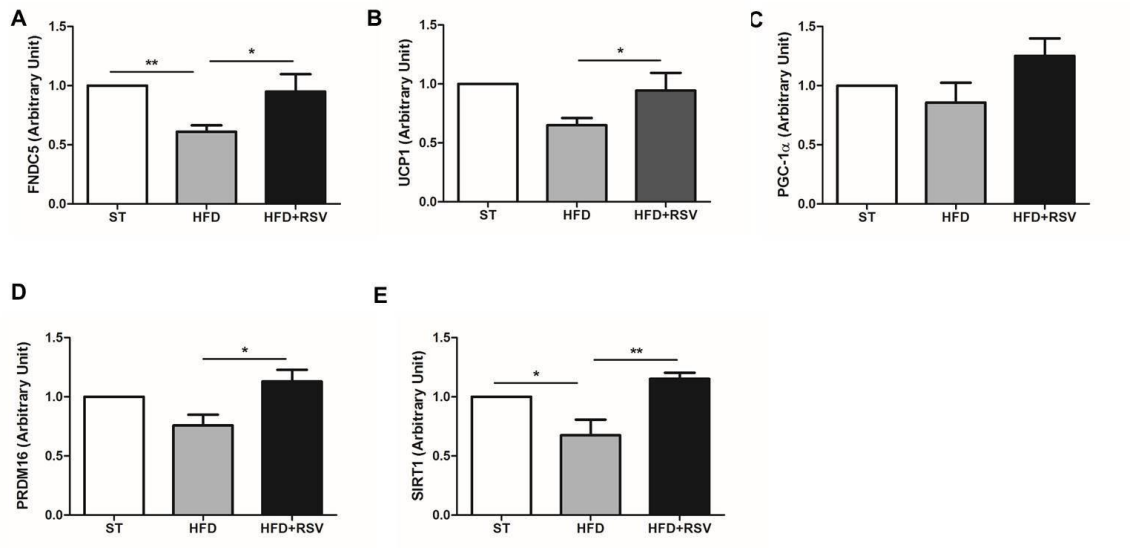

Figure 5. Resveratrol increases expression of FNDC5, SIRT1 and genes involved in brown fat cell differentiation and adaptive thermogenesis in mouse subcutaneous adipose tissue. A) FNDC5 mRNA expression. B) UCP1 mRNA expression. C) PGC-1α mRNA expression. D) PRDM16 mRNA expression. E) SIRT1 mRNA expression. All analysis were made in subcutaneous adipose tissue. Gene expression data were normalized for the expression of GAPDH and gene expression levels in ST group were assumed to be 1. Values are the mean ± SEM (n=6 per group). Differences between groups were analyzed by one-way ANOVA and the Bonferroni *post hoc* test. Data are presented as means ±SEM. **P*<0.05, ***P*<0.01 and ****P*<0.001.

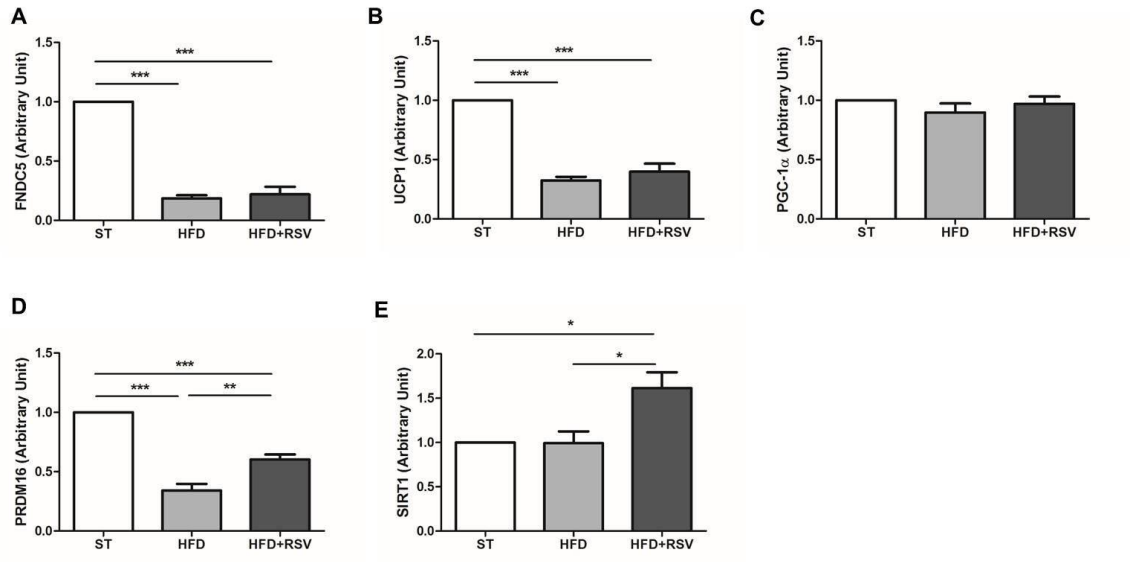

Figure 6. Resveratrol increases expression of FNDC5, SIRT1 and genes involved in brown fat cell differentiation and adaptive thermogenesis in mouse visceral adipose tissue. A) FNDC5 mRNA expression. B) UCP1 mRNA expression. C) PGC-1α mRNA expression. D) PRDM16 mRNA expression. E) SIRT1 mRNA expression. All analysis were made in visceral adipose tissue. All analysis were made in subcutaneous adipose tissue. Gene expression data were normalized for the expression of GAPDH and gene expression levels in ST group were assumed to be 1. Values are the mean ± SEM (n=6 per group). Differences between groups were analyzed by one-way ANOVA and the Bonferroni *post hoc* test. Data are presented as means ±SEM. **P*<0.05, ***P*<0.01 and ****P*<0.001.

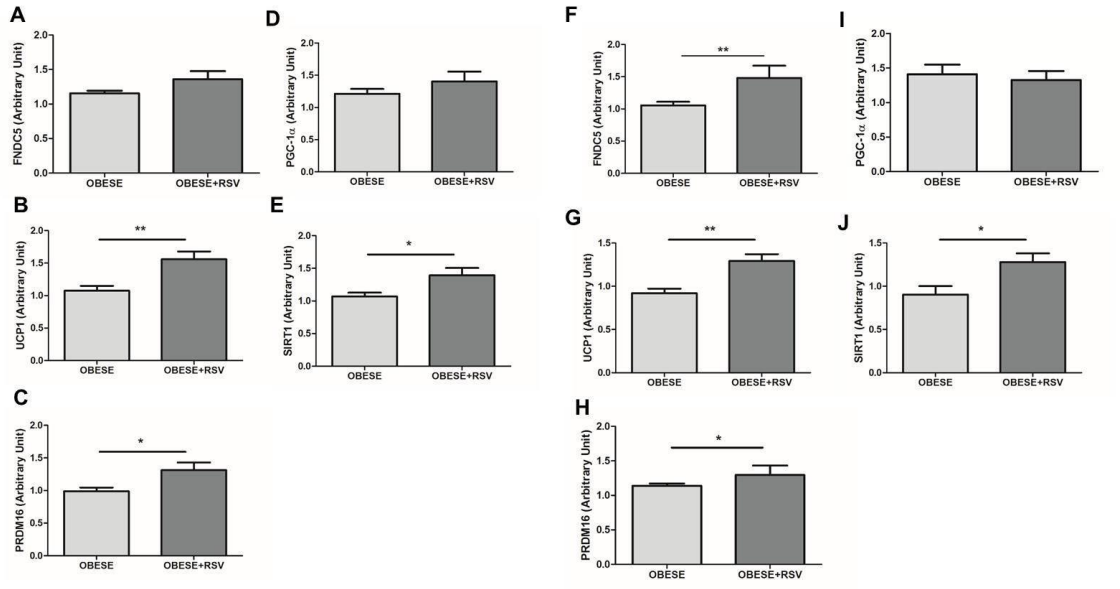

Figure 7. Resveratrol increases expression of FNDC5, SIRT1 and genes involved in brown fat cell differentiation and adaptive thermogenesis in human visceral and subcutaneous adipose tissue. A) FNDC5 mRNA expression. B) UCP1 mRNA expression. C) PRDM16 mRNA expression. D) PGC-1α mRNA expression. E) SIRT1 mRNA expression. All analysis were made in visceral adipose tissue. F) FNDC5 mRNA expression. G) UCP1 mRNA expression. H) PRDM16 mRNA expression. I) PGC-1α mRNA expression. J) SIRT1 mRNA expression. All analysis were made in subcutaneous adipose tissue. Gene expression data were normalized for the expression of GAPDH and gene expression levels in control group were assumed to be 1. Values are the mean ± SEM (n=10 per group). Differences between groups were analyzed by T-student test and the Bonferroni *post hoc* test. Data are presented as means ±SEM. **P*<0.05, ***P*<0.01 and ****P*<0.001.

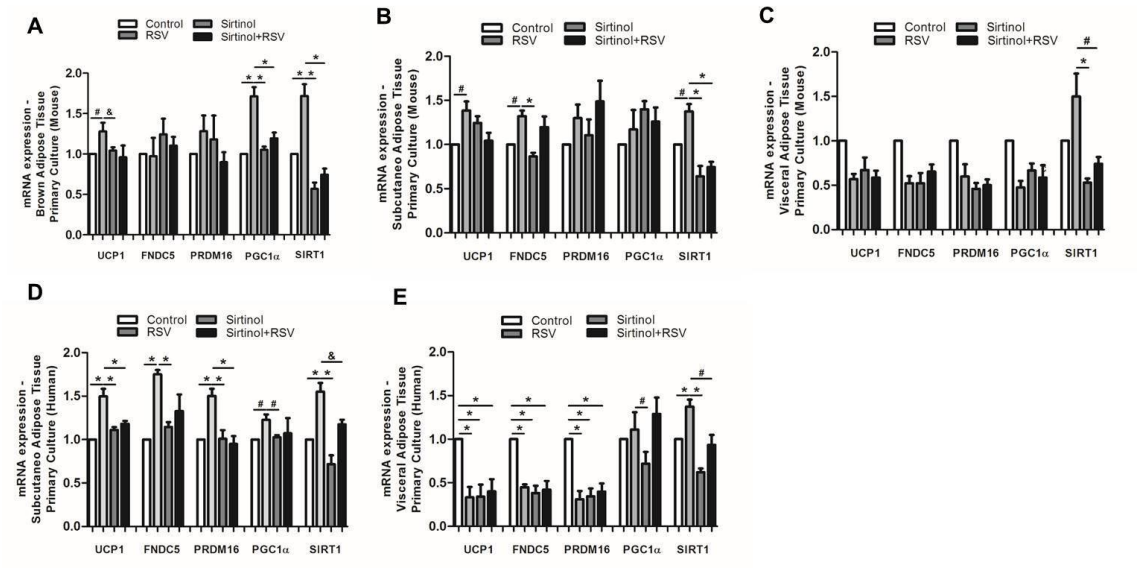

Figure 8. Resveratrol increases expression of FNDC5, SIRT1 and genes involved in brown fat cell differentiation and adaptive thermogenesis in mouse and human primary cell culture adipocytes. A-C) UCP1, FNDC5, PRDM16, PGC-1α and SIRT1 mRNA expression in brown and white mouse primary cell culture adipocytes. D-E) UCP1, FNDC5, PRDM16, PGC-1α and SIRT1 mRNA expression in white human primary cell culture adipocytes. Adipocyte pure cell culture (control), treated with resveratrol (RSV), sirtinol and sirtinol plus resveratrol (sirtinol+RSV). Gene expression data were normalized for the expression of GAPDH and gene expression levels in control group were assumed to be 1. Values are the mean ± SEM (n= 4 per group). Differences between groups were analyzed by one-way ANOVA and the Bonferroni *post hoc* test. Data are presented as means ±SEM. $^{\#}P<0.05$, $^{\&}P<0.01$ and $^{*}P<0.001$.

4 CONSIDERAÇÕES FINAIS

Em resumo, o presente estudo demonstrou que o resveratrol melhora a função metabólica de camundongos alimentados com dietas normo e hiperlipídica, através da diminuição da adiposidade corporal, dos níveis plasmáticos de colesterol, triglicerídeos, leptina e resistina, melhora a sensibilidade insulínica e aumenta o dispêndio energético. Além disso, induz o aumento da expressão nos níveis de marcadores, como FNDC5/irisina, bem como de outros associados à termogênese e ao processo de *browninig* no tecido adiposo de camundongos e humanos. Ademais, os achados indicam pela primeira vez o papel do resveratrol na modulação da expressão de FNDC5/irisina *in vivo* e *in vitro*, em partes devido a ativação da SIRT1.

Em conjunto, esses achados sugerem para o promissor papel do resveratrol na prevenção e tratamento da obesidade e doenças associadas, por meio da modulação do tecido adiposo branco e marrom. Abre novas perspectivas para o campo de estudo em questão, trazendo novas e promissoras evidências que fortalecem o benéfico papel do resveratrol sobre o metabolismo de camundongos e humanos.

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ANEXOS

ANEXO A - Parecer do Comitê de Ética e Bem Estar Animal/UNIMONTES

UNIVERSIDADE ESTADUAL DE MONTES CLAROS
COMISSÃO DE ÉTICA EM EXPERIMENTAÇÃO E BEM-ESTAR ANIMAL

Unimontes

PARECER CONSUBSTANCIADO

Montes Claros, 02 de março de 2012.

Processo N. º **023**
Título do Projeto: **Avaliação Metabólica de Camundongos FVB/N submetidos à Dieta Hiperlipídica e Tratados com Resveratrol e Angiotensina-(1-7).**
Orientador: **Prof. Dr. Sérgio Henrique Sousa Santos**

Histórico
As mudanças ocorridas nos padrões econômicos e culturais, nas últimas décadas, têm alterado de forma significativa o modo de vida das pessoas. Alguns fatores podem estar contribuindo para o aumento da prevalência de doenças crônicas não-transmissíveis, entre os quais destacam-se: hábitos alimentares, estilo de vida e estresse. A síndrome metabólica (SM) é um transtorno complexo representado por um conjunto de fatores de risco cardiovasculares relacionados à deposição central de gordura e à resistência à ação da insulina, sendo um importante fator de risco de mortalidade precoce em indivíduos não-diabéticos e em pacientes com DM tipo 2. Entretanto, o papel da SM como entidade independente e associada a um maior risco para o desenvolvimento de eventos cardiovasculares tem sido recentemente questionado. É pensado que as sirtuínas e a Ang-(1-7) possuem papel primordial na gênese da obesidade e nos problemas decorrentes dela. A evidência para este papel vem do entendimento recente que as sirtuínas e a Ang-(1-7) exercem um papel regulador em vias metabólicas e em processos adaptativos relacionados com a obesidade e os aspectos da síndrome metabólica. Estes incluem a expressão de citocinas pelos adipócitos (adipocinas), a maturação das células de gordura, a secreção de insulina e da sensibilidade dos tecidos, a modulação dos níveis plasmáticos de glicose, colesterol e homeostase lipídica e capacidade de energia mitocondrial. Uma evidência existente, reside sobre o resveratrol, sugerindo que este composto pode ter efeitos sobre as sirtuínas, mediando ações anti-obesidade, como a regulação da homeostase energética, a ingestão alimentar e o peso corporal. Outra ação do resveratrol parece associá-lo a minimização de alguns dos efeitos do alto teor de gordura na dieta, protegendo contra a resistência à insulina, hiperglicemia e dislipidemia. Estudos recentes mostram o Sistema Renina-Angiotensina (SRA) não apenas como um regulador da pressão arterial e homeostasia cardiovascular, mas também como um complexo sistema hormonal envolvido nas mais diversas funções no organismo, relacionadas a processos metabólicos, por meio da atuação da Ang-(1-7). São conhecidas várias interações da Ang-(1-7) com com a função endócrina do tecido adiposo, no entanto, os mecanismos estão pouco compreendidos. Porém não há estudos registrados na literatura científica sobre o efeito da Ang-(1-7) no metabolismo lipídico e glicêmico de roedores submetidos à dieta hiperlipídica quando associada a compostos ativadores das sirtuinas, como o resveratrol por exemplo. Os resultados do presente estudo permitirão a compreensão mais aprofundada dos mecanismos de atuação das sirtuinas e da Ang-(1-7) sobre desordens metabólicas, sendo de fundamental importância como testes pré-clínicos de avaliação de efeitos adversos abrindo a perspectiva de desenvolvimento de novos medicamentos para combater as doenças cardiometabólicas que mais acometem e matam a população mundial.

Mérito
O presente estudo tem como objetivos avaliar o metabolismo glicêmico e lipídico de camundongos da linhagem FVB/N submetidos à dieta hiperlipídica e tratados com resveratrol e angiotensina-(1-7); avaliar a expressão de sirtuinas no tecido adiposo; o padrão de expressão dos marcadores inflamatórios; o padrão de expressão de adipocinas; estudar a regulação glicêmica por meio de testes de sensibilidade insulínica e tolerância a glicose e os níveis glicêmicos durante o jejum; mensurar os níveis plasmáticos lipídicos, dosando os níveis de triglicérides, colesterol total e HDL e comparar a histologia dos adipócitos entre grupos de animais. Os grupos experimentais terão a dieta hiperlipídica adicionadas de doses de Angiotensina-(1-7) e/ou Resveratrol por cerca de 6 a 8 semanas.

Parecer
A Comissão de Ética em Experimentação e Bem-Estar Animal da Unimontes analisou o processo **023** e entende que o mesmo está completo e dentro das normas da Comissão e das Resoluções do Conselho Nacional de Controle e Experimentação Animal. Sendo assim, somos pela **APROVAÇÃO** do projeto de pesquisa.

Prof. Orlando Raphael Lopasso Júnior
Presidente da Comissão de Ética em Experimentação e Bem-Estar Animal da UNIMONTES