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FILIPY BORGHI RODRIGUES DE SOUZA

CARACTERIZAÇÃO DO PERFIL METABÓLICO DO TECIDO ADIPOSO DE RATOS ESPONTANEAMENTE HIPERTENSOS E O SEU ENVOLVIMENTO COM O SISTEMA RENINA-ANGIOTENSINA-ALDOSTERONA

CHARACTERIZATION OF THE METABOLIC PROFILE OF ADIPOSE TISSUE IN SPONTANEOUSLY HYPERTENSIVE RATS AND ITS INVOLVEMENT WITH THE RENIN-ANGIOTENSIN-ALDOSTERONE SYSTEM

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Orientador: Profª. Drª. Dora Maria Grassi Kassisse

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If you surrender to the air, you can ride it

Toni Morrisson, Howard University 1953

RESUMO

O estresse crônico está associado a distúrbios cardiovasculares e metabólicos. A ativação crônica do eixo hipotálamo-hipófise-adrenal, do sistema nervoso simpático (SNS) e, consequentemente, do sistema renina-angiotensina-aldosterona (SRAA) tem papel crucial no desenvolvimento da hipertensão e de doenças metabólicas. Além de presente em diferentes tecidos, já foi comprovado que o adipócito produz todos os elementos do SRAA, envolvendo o eixo adipócito-corticomedular diretamente com a obesidade. O objetivo deste trabalho foi caracterizar o perfil metabólico do tecido adiposo em ratos espontaneamente hipertensos (SHR) e o seu envolvimento com o sistema renina-angiotensina-aldosterona. O peso corpóreo, ingestão alimentar e hídrica, coleta de sangue para análises séricas e de tecidos para morfometria e FLIM (Fluorescence-lifetime imaging microscopy) foram realizadas na 6ª e na 15ª semana de vida. Os ensaios funcionais foram realizados com adipócitos epididimais isolados de ratos submetidos a jejum prévio de 12-16h na 15ª semana de vida. Os SHR apresentaram valores menores no peso corporal e na glicemia de jejum, além de apresentarem uma taxa basal maior de produção de lactato por volume de adipócitos. A estimulação por angiotensina II (AII) e o bloqueio de seus receptores não interferiram na produção de lactato. A estimulação de noradrenalina e sua combinação com AII sugere uma modulação e/ou competição na cascata downstream para a troca do metabolismo glicolítico pelo lipolítico em todas as linhagens, demonstrando que a hipolipodristrofia exibida em SHR não é ocasionada por nenhum distúrbio nestas vias. Além disso, demonstramos que o fenômeno do whitening está associado com mudanças ocasionadas pela idade, podendo ser moduladas pelo eixo Hipotálamo-Hipófise-Tireóide (TRH-T3). Desta forma, o ambiente hormonal proporcionado pelo desenvolvimento da hipertensão pode destacar moléculas-chave que podem ser o caminho para o desenvolvimento de abordagens terapêuticas inovadoras para o tratamento de disfunções da adiposidade e hipertensão, além de esclarecer o entendimento sobre a relação causal entre hipolipodistrofia e hipertensão.

Palavras Chave: Adipócitos; FLIM; glicerol; lactato; sistema renina-angiotensina-aldosterona; SHR; UCP-1.

ABSTRACT

Chronic stress is associated with cardiovascular and metabolic disorders. The chronic activation of the hypothalamic-pituitary-adrenal axis, the sympathetic nervous system (SNS) and, consequently, the renin-angiotensin-aldosterone system (RAAS) plays a crucial role in the development of hypertension and metabolic diseases. It has been proven that the adipocyte produces all elements of the RAAS, involving the adipocyte-corticomedullary axis directly to obesity. The objective of this study was to characterize the metabolic profile of adipose tissue in spontaneously hypertensive rats (SHR) and its involvement with the renin-angiotensin-aldosterone system. Body weight, food and water intake, blood collection for serum and tissue analyzes for morphometry and FLIM (Fluorescence-lifetime imaging microscopy) were performed at the 6th and 15th week of life. Functional assays were performed with epididymal isolated adipocytes from rats submitted to a previous fast of 12-16h in the 15th week of life. The SHR presented lower values for body weight and fasting blood glucose, in addition to presenting a higher basal rate of lactate production per adipocyte volume. Angiotensin II (AII) stimulation and blockade of its receptors did not interfere with lactate production. Norepinephrine stimulation and its combination with AII suggests a modulation and/or competition in the downstream cascade for the exchange of glycolitic to lipolytic metabolism in all strains, demonstrating that the hypolodystrophy exhibited by SHR is not caused by any disturbance in these pathways. Moreover, we demonstrated that the whitening phenomenon is associated with changes caused by age and can be modulated by the Hypothalamic-Pituitary-Thyroid (TRH-T3) axis. Thus, the hormonal environment provided by the development of hypertension may highlight key molecules that may be the pathway for the development of novel and innovative therapeutic approaches for the treatment of adiposity dysfunctions and hypertension, in addition to clarify the understanding about the causal relationship between hypolipodystrophy and hypertension.

Keywords: Adipocytes; FLIM; glycerol; lactate; renin-angiotensin-aldosterone system; SHR; UCP-1.

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AC – Adenilato ciclase

ACTH – Hormônio adrenocorticotrópico

AI – Angiotensina I

AII – Angiotensina II

aP2 – Complexo proteico adaptador

ATP – Trifosfato de adenosina

AT1 – Receptor de angiotensina II tipo 1

AT2 – Receptor de angiotensina II tipo 2

BAT – Tecido adiposo marrom

cAMP – Monofosfato cíclico de adenosina

C/EBPα – Proteína alfa potenciadora de ligação CCAAT

CRH – Hormônio liberador de corticotropina

ECA/ACE – Enzima conversora de angiotensina

ERK – quinase regulada por sinal extracelular

eWAT – Tecido adiposo branco epididimal

FAD – Dinucleótido de Flavina e Adenina

FLIM – Fluorescence-lifetime imaging microscopy

GLUT-4 – Transportador de glicose tipo 4

HPA – Eixo Hipotálamo-hipófise-adrenal

HHP/HPT – Eixo Hipófise-hipotálamo-tireoide

HSL – Lipase hormônio sensível

IL-6 – Interleucina 6

IP3 – Inositol trifosfato

iWAT – Tecido adiposo branco inguinal

JAK – Janus quinase

LDH – Lactato desidrogenase

LPL – Lipoproteína lipase

LOS – Losartana potássica

MAPK – Proteíno-quinases ativadas por mitógenos

MCP-1 – Proteína quimiotática de monócitos 1

R_{max} – Resposta máxima

R_{min} – Resposta mínima

MR – Receptor mineralocorticoide

mWAT – Tecido adiposo branco mesentérico

NADH – Nicotinamida Adenina Dinucleotídeo

Nor – Noradrenalina

PD – PD 123319

PLC – Fosfolipase C

PPAR-γ – Receptor ativado por proliferadores de peroxissoma gama

RNAm – Ácido ribonucleico mensageiro

rWAT – Tecido adiposo branco retroperitonial

SHR – Rato espontâneamente hipertenso

SNS – Sistema Nervoso Simpático

SRAA/RAAS – Sistema renina-angiotensina-aldosterona

STAT – Transdutores de sinal e ativadores da transcrição

T3 – Triiodotironina

TA/AT – Tecido adiposo

TNFα – Fatores de Necrose Tumoral Alfa

TRH – Hormônio liberador de tireotrofina

TSH – Hormônio estimulante da tiroide

UCP-1 – Proteína de desacoplamento tipo 1

WAT – Tecido adiposo branco

WIS – Wistar

WKY – Wistar-Kyoto

SUMÁRIO

INTRODUÇÃO

A sobrevivência de organismos complexos depende da manutenção da alostase, uma condição dinâmica de equilíbrio baseada em *set points* (pontos de ajustes), onde os critérios de resposta estão em constante mudança. Diferentes agentes desencadeadores da resposta de estresse provocam distintos padrões de ativação principalmente no sistema nervoso simpático (SNS), nos hormônios adrenomedulares e no eixo hipotálamo-hipófise-adrenal (HPA) fechando a alça de feedback negativo. O eixo HPA está estritamente ligado à função do tecido adiposo tanto nas condições fisiológicas quanto nas patológicas, embora para muitos aspectos existam mais hipóteses do que um cenário claro (Vicennati et al. 2014).

Fisiologia da reação ao estresse

Os estímulos identificados como ameaça ao organismo são transmitidos via tronco cerebral até o córtex e em seguida ao hipotálamo, que induzirá o aumento na liberação do hormônio liberador de corticotropina (CRH). No hipotálamo, também ocorre o estímulo do eixo HPA com consequente ativação do SNS. Em questão de segundos a minutos, ocorre aumento nas concentrações circulantes de hormônio adrenocorticotrópico (ACTH), que desencadeia aumento na secreção na região cortical da glândula adrenal de hormônios esteroides como: mineralocorticoides, glicocorticoides e androgênios (Charmandari, Tsigos and Chrousos 2005). A ativação do SNS via ACTH na região medular da glândula adrenal, por sua vez, resulta em aumento nas concentrações circulantes de catecolaminas (Goldstein 2003). A inter-relação entre hipotálamo, SNS, adenohipófise, córtex e medula da adrenal foi enfatizada por Axelrod e Reisine, que classificaram como hormônios do estresse, além do ACTH e dos glicocorticoides, a adrenalina e a noradrenalina (Axelrod and Reisine 1984). A combinação das secreções dos eixos hipófise-adrenal e simpático-adrenal constituiria a resposta neuroendócrina aos estímulos estressores (Goldstein 2003, Sapolsky, Romero and Munck 2000). No estresse agudo há predomínio da ativação medular do SNS, enquanto no estresse crônico

a via cortical é a mais prevalente, alterando assim as concentrações e ritmicidade na produção hormonal.

Os efeitos do estresse podem se manifestar em quatro domínios distintos: fisiológico, comportamental, experiência subjetiva e função cognitiva. Os efeitos fisiológicos incluem alterações no sistema neuroendócrino, sistema nervoso autonômico e sistema imunológico. Estes sistemas alterados evocam mudanças adaptativas em praticamente todo o organismo. Todas estas alterações resultam em diferentes respostas de ativação dos eixos HPA e SNS adrenomedular (Steptoe 2000).

As catecolaminas, também presentes na alostasia e na sobrecarga alostática, atuam na disponibilização de energia armazenada para ser utilizada nas situações de ameaça à integridade física. Além de seus efeitos diretos no sistema cardiovascular, elas têm importante ação na ativação da produção e liberação de renina e consequentemente de todo o Sistema Renina-Angiotensina-Aldosterona (SRAA). O SRAA tem como principal função coordenar os ajustes cardiovasculares e endócrinos para a manutenção da pressão arterial em longo prazo e equilíbrio hidroeletrolítico (Hökfelt 1978, Borghi, Sevá-Pessôa and Grassi-Kassisse 2016).

As correlações diretas ou indiretas de distúrbios decorrentes de estresse agudo e crônico são grandes desafios. Assim, o estudo de distúrbios relacionados à reação ao estresse crônico e sobrecarga alostática em constante ascensão na sociedade atual, como metabólicos, cardiovasculares, autoimunes e na reprodução, é altamente relevante, e investigações que correlacionam dois ou mais destes sistemas são desafiadoras.

Sistema Renina-Angiotensina-Aldosterona

O SRAA, além de estar envolvido com a manutenção da pressão arterial em longo prazo e com o equilíbrio hidroeletrolítico, contribui para a produção de vasopressina e modula o tônus do sistema nervoso autonômico, mais especificamente o simpático (Paulis and Unger 2010). A via clássica do SRAA se inicia com a ativação do receptor de pró-renina, transformando a renina em

sua forma ativa, que cliva o angiotensinogênio em angiotensina I (AI). A AI, por sua vez, sofre ação da enzima conversora de angiotensina (ECA), sendo clivada e transformada em angiotensina II (AII). A AII é um potente agente vasoconstritor e induz a liberação da aldosterona no córtex da adrenal e de vasopressina, ou hormônio antidiurético, centralmente (Paulis and Unger 2010, Borghi et al. 2016). Na visão clássica do SRAA, a produção excessiva de AII, atuando no seu receptor AT1, é a principal responsável pela maioria dos efeitos deletérios, tais como hipertensão, disfunção endotelial e fibrose. Ela também é responsável pelo aumento da volemia e da pressão arterial devido à retenção de sódio e água causada pela aldosterona e hormônio antidiurético, respectivamente (Sevá Pessôa et al. 2013).

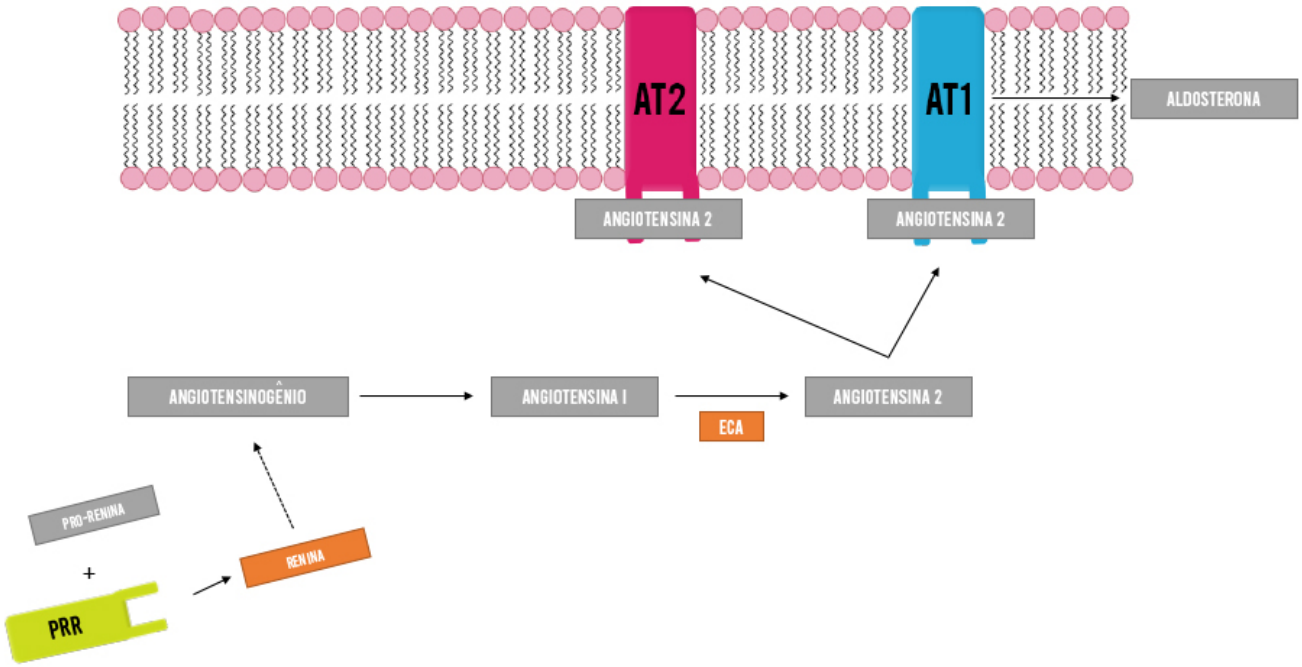

Figura 1 – Esquema da ativação do sistema renina-angiotensina-aldosterona.

A utilização de bloqueadores deste sistema tem contribuído para a longevidade de muitos pacientes hipertensos e contribui para o melhor entendimento desse sistema (Briasoulis and Bakris 2013). A síntese da AII pode também ser estimulada pelo ACTH, noradrenalina, endotelina e pela serotonina, além de ser inibida pelo peptídeo natriurético atrial e o óxido nítrico (Atlas 2007, El Ghorayeb, Bourdeau and Lacroix 2016). Embora a abordagem terapêutica

para o tratamento da hipertensão tenha avançado considerando alterações fisiopatológicas no SRAA, no momento, ainda há pacientes que não respondem de forma eficiente ao controle da hipertensão (Ikeda et al. 2014, McManus et al. 2018).

Desde que Engeli et al. (Engeli et al. 2003) demonstraram que o SRAA está localizado em muitos tecidos, incluindo o tecido adiposo, vários estudos surgiram para investigar o papel deste sistema na diferenciação dos adipócitos e na hipertensão. Neste trabalho os autores demonstraram que a superalimentação de roedores leva ao aumento da formação local de AII devido a um aumento da secreção de angiotensinogênio pelos adipócitos. A AII exerce efeito antilipolítico direto em adipócitos isolados pela atuação em receptores AT1 (Chaves, Frasson and Kawashita 2011). Esta ligação gera vários sinais, com efeitos em segundos (via PLC e geração de IP3 e liberação de cálcio), em minutos (via ativação da MAPK) e em horas (via ativação da via JAK e Stat) (Boschmann et al. 2006b, Goossens et al. 2007, Goossens et al. 2004, Engeli et al. 2018). Por outro lado, Cabassii e colaboradores demonstraram em ensaios in vivo o efeito lipolítico da AII sistêmica por meio da ativação adrenérgica (Cabassi et al. 2005, Winkler et al. 2018). Complementarmente, outros trabalhos demonstram que os receptores AT1 e AT2 estão envolvidos na determinação do tamanho do adipócito (Yvan-Charvet et al. 2005, Zorad et al. 2006, Shum et al. 2013).

Zorad e colaboradores demonstraram que a utilização de antagonista de AT1 ao longo de 18 semanas em ratos Wistar-Kyoto (WKY) provocou queda de peso corporal e diminuição do panículo adiposo epididimal e retroperitoneal, diminuindo também o tamanho dos adipócitos, sem, portanto, alterar o número de adipócitos. A linha de investigação foi a quantificação sérica de leptina, adiponectina e a expressão de RNAm da adiponectina, do TNFα, e a síntese de ácidos graxos. Ao final do período experimental houve diminuição nos valores séricos de leptina e do RNAm do TNFα e na expressão do receptor de renina e aumento nos valores séricos e no RNAm da adiponectina, síntese de RNAm de ácidos graxos e aumento na expressão do RNAm do PPAR-γ epididimal e do receptor de AII. Estes autores demonstram a importante atividade do SRAA no

metabolismo do tecido adiposo, e sugerem mecanismos adicionais à inibição da AII (Zorad et al. 2006, Pahlavani et al. 2017).

Outro componente do SRAA, a aldosterona, também atua em adipócitos. Foi descrito por diferentes investigadores uma correlação positiva entre as concentrações de aldosterona no plasma e a obesidade, sugerindo que uma secreção anormal de aldosterona contribui para o desenvolvimento desta doença (Briones et al. 2012, Calhoun and Sharma 2010, Dall'Asta et al. 2009). Os mecanismos propostos para explicar esta produção de aldosterona anormal envolvem principalmente o SRAA do adipócito, um efeito indireto do aumento dos ácidos graxos e estimulação da adrenal diretamente por adipocinas. A atividade estimulante de mineralocorticoide foi observada mais recentemente em adipócitos isolados de humanos, sugerindo um envolvimento direto, até então desconhecido do tecido adiposo, na regulação da pressão arterial na obesidade (Briones et al. 2012, Lamounier-Zepter, Ehrhart-Bornstein and Bornstein 2005, Faulkner, Bruder-Nascimento and Belin de Chantemèle 2018).

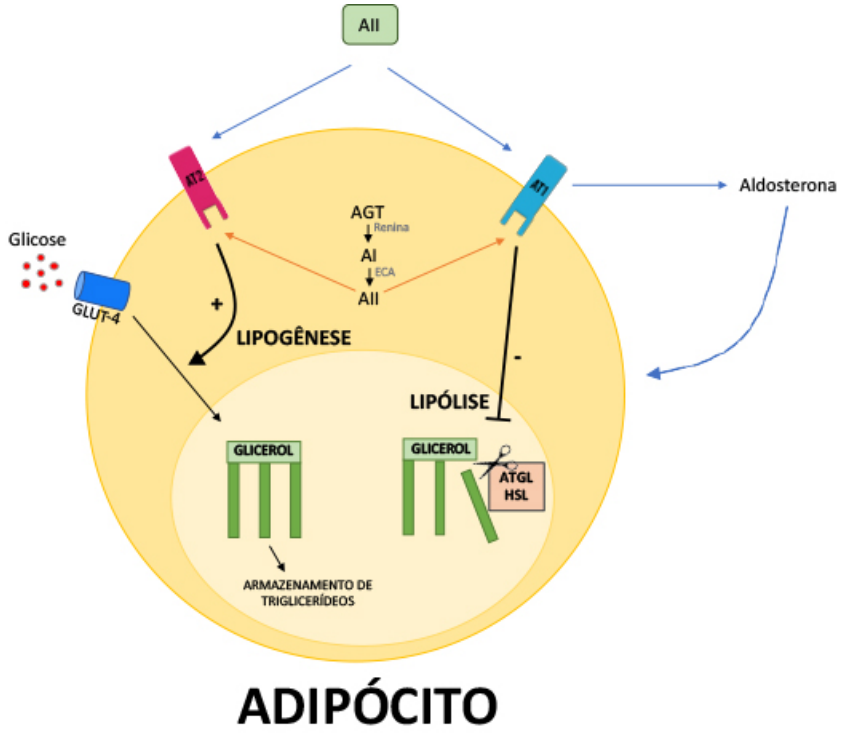

Figura 2 – Atuação dos componentes do sistema renina-angiotensina-aldosterona no adipócito.

A coexistência dessas duas patologias é frequentemente descrita na literatura, com muitos artigos indicando que os obesos se tornam hipertensos ou vice-versa. Nestes casos, o estresse crônico é comumente associado ao sedentarismo, associando esta hipertensão a fatores ambientais. No entanto, a hipertensão associada à genética, muito comum em nossa sociedade, apresenta relação inversa entre hipertensos e adiposidade. Nesse caso, indivíduos magros apresentam valores de pressão arterial mais elevados associados a altas concentrações lipidêmicas devido à falta de depósitos adiposos para armazenar os triacilglicerídeos obtidos pela dieta (Vernochet et al. 2014, Wu et al. 2016).

Condições hormonais na hipertensão

No Brasil, a hipertensão afeta 30% da população (Picon et al. 2012, Picon et al. 2017). Doenças cardiovasculares são as principais responsáveis pelas mortes no mundo e a maioria é decorrente da hipertensão (Perk et al. 2012). Para o estudo desta doença existem vários modelos animais que contribuem para gerar conhecimento e elucidar os mecanismos complexos envolvidos com esta doença (Bernatova 2014). Um dos modelos para o estudo da hipertensão são os ratos espontaneamente hipertensos (SHR), que desenvolvem um quadro multifatorial extremamente semelhante à hipertensão essencial em humanos (Dornas and Silva 2011, Conceicao-Vertamatti et al. 2017, Leong, Ng and Jaarin 2015, Pravenec et al. 2014). Este modelo foi desenvolvido por Okamoto e Aoki em 1963 e seu controle é denominado de Wistar-Kyoto (WKY) (Okamoto and Aoki 1963).

A participação do SNS e de hormônios corticoides e mineralocorticoides em SHR foram descritas em condições basais já na década de 70. Sabe-se que o desenvolvimento da hipertensão em SHR é caracterizado pelo aumento na atividade simpatoadrenal que acontece entre o 1º e 3º mês de idade, que é seguida pelo aumento na pressão arterial (Hökfelt 1978, Judy et al. 1978, Kvetnansky et al. 1979). As concentrações plasmáticas de aldosterona e de corticosterona estão elevadas em SHR quando comparados a outras espécies de ratos sob condições basais (Iams, McMurthy and Wexler 1979, Sowers et al.

1980, Sowers et al. 1981). Entretanto, dados mais recentes do grupo de Berg indicam valores aumentados de catecolaminas em SHR sem, portanto, apresentarem diferenças significativas em relação ao seu controle WKY (Berg 2014, Berg 2015, Berg 2016).

Estudos demonstraram a participação do SRAA na hipertensão de ratos espontaneamente hipertensos principalmente seguindo protocolos experimentais que utilizam bloqueadores ou inibidores enzimáticos de um ou mais componentes desta via (Baumann et al. 2007, Berecek, Nagahama and Oparil 1984, Harrap et al. 1990, Klimas et al. 2012, Kristek, Malekova and Cacanyiova 2013, Regan, Bishop and Berecek 1997, Lezama-Martínez et al. 2017).

O tecido adiposo e as lipoatrofias

A epidemia de obesidade intensificou esforços para compreensão dos mecanismos que controlam o desenvolvimento do tecido adiposo, desta forma estudos envolvendo a embriogênese do tecido adiposo (TA), a sua distribuição corporal, bem como sua regulação, são focos de várias pesquisas nos últimos anos (Sanchez-Gurmaches and Guertin 2014, Lee, Wu and Fried 2013). Sabe-se também que o padrão de distribuição de gordura corporal é altamente variável entre os indivíduos e que existem diferenças importantes entre os depósitos regionais, alguns apresentando propriedades metabólicas mais favoráveis do que os outros (Ma et al. 2015, Lee et al. 2013, Sanchez-Gurmaches and Guertin 2014, Farkas et al. 2018).

O TA é o único tecido com potencial crescimento, praticamente ilimitado, em qualquer estágio da vida (Pellegrinelli, Carobbio and Vidal-Puig 2016). Ao mesmo tempo pode reduzir seu tamanho em situações de desnutrição e em programas de emagrecimento, com recidiva de recuperação de seu tamanho se a dieta for interrompida (MacLean et al. 2015).

A literatura é vasta com históricos e revisões a respeito das diferentes funções do TA. Uma das principais funções dos adipócitos é armazenar os ácidos graxos durante o excedente de energia e liberá-los durante o jejum ou

inanição (Scherer 2006, Lelliott and Vidal-Puig 2004, Carobbio, Pellegrinelli and Vidal-Puig 2017). No entanto, inúmeras funções adicionais foram descritas trazendo o tecido adiposo para a categoria de um órgão endócrino com grande atividade secretora (Scherer 2006, Zhu and Scherer 2018).

Os adipócitos interagem com outros tecidos metabolicamente ativos por meio da produção e liberação de fatores conhecidos como adipocinas (Cook et al. 1987, Zhang et al. 1994, Scherer et al. 1995, Hu, Liang and Spiegelman 1996, Maeda et al. 1996, Nakano et al. 1996, Scherer 2006, Zhu and Scherer 2018) e de citocinas pró-inflamatórias que estão presentes em baixas quantidades no adipócito saudável (Scherer 2006, Lin et al. 2001, McNamara and Huber 2018). O TA tem uma importante plasticidade (Pellegrinelli et al. 2016, Murawska-Ciałowicz 2017) e é geralmente classificado como branco (WAT), o principal tecido de armazenamento de energia, ou marrom (BAT), que medeia a termogênese. A denominação bege/brite é caracterizada quando um específico fenótipo de tecido adiposo se diferencia em outro. Desta maneira, podemos observar o adipócito marrom adquirindo o fenótipo branco, compreendendo o fenômeno denominado whitening (Hill 2015, Peirce, Carobbio and Vidal-Puig 2014) ou o adipócito branco adquirindo o fenótipo marrom, caracterizando o browning (Wang et al. 2016, Peirce et al. 2014, Sepa-Kishi and Ceddia 2018).

Assim como os adipócitos uniloculares brancos presentes no WAT, os adipócitos multiloculares marrons presentes do BAT também acumulam e armazenam lipídios. No entanto, os adipócitos marrons são diferentes dos adipócitos brancos pela presença de grandes quantidades de UCP-1, que atua como um canal de próton, dissipando o gradiente eletroquímico de prótons gerados na respiração na forma de calor ao invés de formar trifosfato de adenosina (ATP) (Peirce et al. 2014, Cannon and Nedergaard 2004). O fato de a produção de calor pelo BAT ser um processo com alto gasto energético e, que utiliza substratos energéticos, tem duas ressalvas importantes. Em primeiro lugar, para evitar a hipertermia e regular o consumo de energia pelo BAT, a termogênese neste tecido deve ser rigorosamente controlada de modo que o calor seja produzido especificamente em resposta a estímulos termogênicos. Em segundo lugar, quando estimuladas para produzir calor, o chamado BAT ativado

tem um enorme impacto sobre o equilíbrio de energia, pelo menos em roedores. Por exemplo, camundongos alojados a temperatura ambiente de 20-22°C necessitariam consumir mais 60% de alimento para manter o seu peso corporal para compensar o aumento do gasto de energia como resultado de um aumento termogênico, em comparação com camundongos alojados a temperaturas consideradas termoneutras (30°C) (Cannon and Nedergaard 2004, van der Stelt et al. 2017). Por outro lado, mesmo em termoneutralidade, a ablação ou disfunção de BAT em roedores diminui o gasto de energia, causando o fenótipo de obesos (Feldmann et al. 2009, Lowell et al. 1993, Sellayah and Sikder 2014).

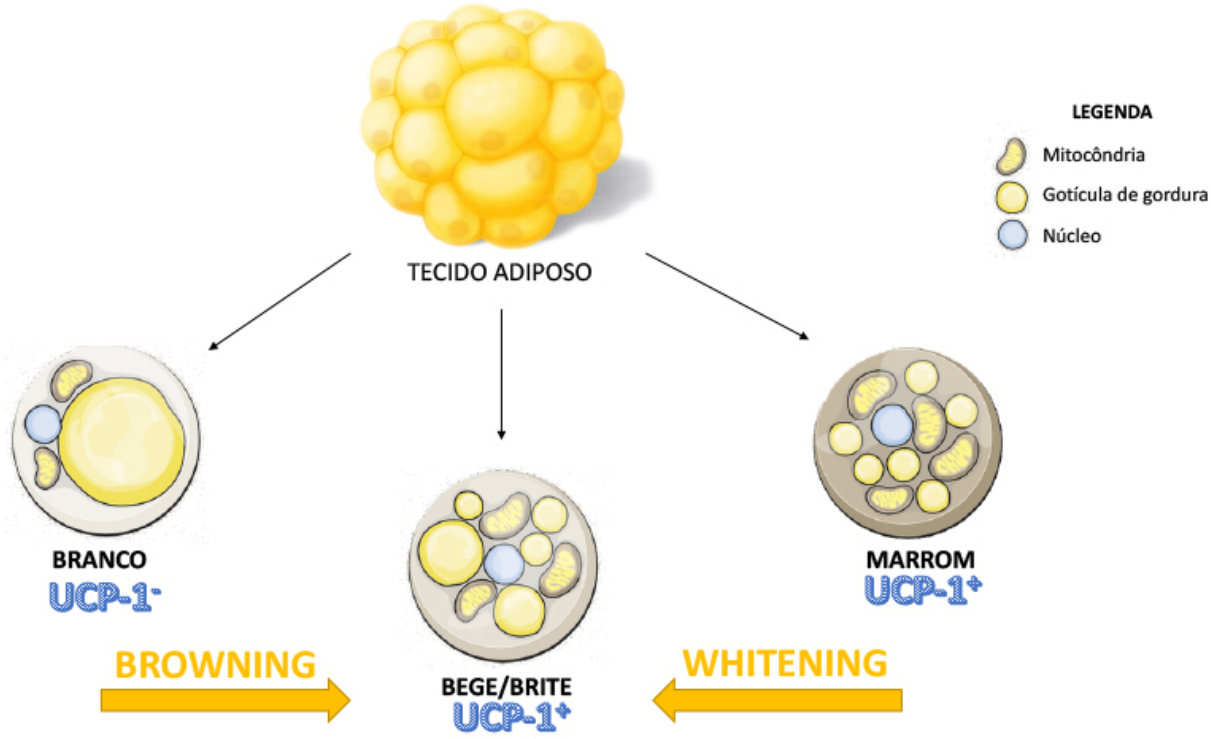

Figura 3 – O tecido adiposo e suas variações.

Em adição aos seus efeitos termogênicos, a ativação de BAT tem uma grande capacidade de absorção de glicose e de lipídios por grama de tecido, tal como demonstrado pela sua capacidade para normalizar a hiperglicemia e hiperlipidemia em modelos de rato de diabetes e de dislipidemia (Bartelt et al. 2011, Arbeeny et al. 1995). Em linha com sua notável capacidade de oxidação de substrato, o BAT é ativado em roedores em resposta ao consumo de nutrientes em excesso, como a ingestão de dieta rica em gordura, um processo

conhecido como termogênese induzida por dieta (Rothwell and Stock 1983, Okla et al. 2017). Portanto, embora WAT seja muito superior à BAT em termos de massa percentual corporal, o BAT ativado é um importante contribuinte para a utilização de nutrientes e consequente regulação do peso corporal (Peirce et al. 2014, Marlatt and Ravussin 2017).

A informação de que a gordura marrom existe em humanos adultos e a possibilidade do aumento do gasto energético pelo tecido poder ser uma estratégia terapêutica para combater a obesidade incitou pesquisadores a se envolverem em novas vias de investigação (Sanchez-Gurmaches and Guertin 2014). Dos estudos relacionados ao desenvolvimento do TA, partindo de seu precursor adulto e ancestral embrionário, emergiu um modelo de adipócitos marrons que se originam a partir de um precursor comum às células do músculo esquelético que expressam Myf5, enquanto todos os adipócitos brancos são originários de um precursor negativo para Myf5. Embora tal informação tenha proporcionado uma explicação racional para o fato de o BAT ser metabolicamente mais favorável do que o WAT, trabalhos recentes indicam que a situação é mais complexa, dada a presença de subconjuntos de adipócitos brancos que podem surgir a partir de precursores Myf5-Cre positivos. Desta forma, a identidade da célula progenitora do adipócito está em debate. A diferença de origem entre adipócitos poderia explicar a heterogeneidade metabólica entre os diferentes depósitos de gordura e como as diferentes distribuições de gordura influenciam particularmente em distúrbios como a lipoatrofia (Sanchez-Gurmaches and Guertin 2014).

As lipoatrofias são distúrbios relacionados às condições anormais ou degenerativas dos depósitos de gordura corporal (Herranz et al. 2008, Adams et al. 2018). Algumas lipoatrofias presentes em distúrbios com distribuição regional de gordura são caracterizadas também pela expansão de alguns depósitos de gordura (Garg 2004, Herbst 2012, Adams et al. 2018). Entretanto, apesar de pouco estudada, sabe-se que a perda excessiva ou ausência de determinados panículos adiposos está associada a graves distúrbios como resistência à insulina (Gorden and Gavrilova 2003, Moitra et al. 1998, Pajvani et al. 2005), dislipidemias, hipertensão (Vernochet et al. 2014, Wu et al. 2016) e disfunções

na fertilidade, já que esta última está atrelada à queda na síntese de estrógenos e andrógenos pelos adipócitos (Cong et al. 2016).

As consequências da lipoatrofia, pelo menos em parte, são devidas à ausência de um compartimento especializado na estocagem de lipídios em condições normais. Isto leva a desregulação nas concentrações séricas normais de triacilgliceróis e ácidos graxos livres, bem como na produção de adipocinas (Scherer 2006). Alguns modelos lipodistróficos já foram descritos na literatura. Modelos descritos por Bruce Spiegelman, Brown e Goldstein e os camundongos A/ZIP descritos por Vinson e Reitman, trazem informações relevantes do papel fisiológico do tecido adiposo (Ross, Graves and Spiegelman 1993, Shimomura et al. 1998, Moitra et al. 1998). Entretanto, todos estes modelos têm a desvantagem de serem lipoatróficos, ou seja, não apresentam depósito de gordura durante o desenvolvimento, o que resulta em severa resistência à insulina e esteatose hepática na juventude (Scherer 2006, Adams et al. 2018).

SRAA e tecido adiposo - a relação cardiometabólica

Os mineralocorticoides desempenham um importante papel na diferenciação de células de gordura branca e marrom. Em pré-adipócitos marrons, a ativação dos receptores MR aumenta a expressão de genes adipogênicos como LPL, PPAR-γ e aP2, induzindo acúmulo de gotículas de lipídios intracitoplasmáticas e promove a diferenciação celular. A aldosterona inibe a expressão e função UCP-1, promovendo, assim, o processo de diferenciação do adipócito, e impedindo a termogênese. Em pré-adipócitos brancos, a ativação de MR induz a proliferação celular e, posteriormente, desencadeia a maquinaria transcricional adipogênica, aumento na expressão de genes C/EBPα e PPAR-γ, com subsequente aumento intracelular de triacilgliceróis e expressão de adipocinas. Além disso, a ativação de MR induz a expressão de genes pró-inflamatórios, tais como TNF-α, IL-6 e MCP-1, favorecendo a infiltração de macrófagos e proporcionando as condições ambientais para a inflamação (Marzolla et al. 2012, Infante et al. 2017). Estes resultados adicionam novas informações para os fatores liberadores de

mineralocorticoides produzidos por adipócitos, que estimulam a esteroidogênese em células adrenocorticais (Ehrhart-Bornstein et al. 2004, Lamounier-Zepter et al. 2005, Nagase et al. 2006, Schinner et al. 2007, Infante et al. 2017).

Os adipócitos também produzem aldosterona, que participa na formação de lipídeos e na diferenciação de adipócitos. Curiosamente, a contribuição da AII parece ser diferente na produção de aldosterona pelo adipócito e na atividade liberadora de aldosterona em células adrenais, destacando um papel complexo da AII no eixo célula adipócito-adrenal e sua contribuição para a produção de aldosterona (Briones et al. 2012, Szczepanska-Sadowska, Czarzasta and Cudnoch-Jedrzejewska 2018). A AII produzida localmente ou sistemicamente regula a secreção de aldosterona a partir de adipócitos por meio da calcineurina e do fator nuclear das células T ativadas. Na obesidade, a superexpressão da aldosterona sintase (CYP11B2) promove maior produção de aldosterona. Assim, a aldosterona derivada dos adipócitos pode contribuir para aumentar a adipogênese e a hipertrofia dos adipócitos de maneira autócrina. Por outro lado, de maneira parácrina, tanto a aldosterona como os glicocorticoides produzidos por adipócitos ativam receptores MRs vasculares que participam da disfunção endotelial associada à obesidade (Briones et al. 2012, Kawarazaki and Fujita 2016).

Desta forma, estudos têm demonstrado o envolvimento direto do tecido adiposo no desenvolvimento da hipertensão, por meio da produção aumentada de aldosterona pela glândula adrenal, que por sua vez, vai intensificar o aumento da pressão arterial e a disfunção endotelial seguidas de inflamação (Farquharson and Struthers 2002, Caprio et al. 2008, Faulkner et al. 2018). Por outro lado, as concentrações aumentadas de aldosterona produzidas e secretadas pelas células adrenocorticais, podem induzir a diferenciação, expansão e o processo inflamatório, sustentando a alça regulatória entre tecido adiposo e adrenal.

Função metabólica do tecido adiposo

A unidade funcional do tecido adiposo tem sido extensamente estudada, considerando principalmente seu papel endócrino que ganha funções novas a cada dia (Lee 2018, Zhu and Scherer 2018, Schoettl, Fischer and Ussar 2018). Entretanto, a sua função metabólica perdeu interesse ao longo do tempo devido à grande inespecificidade de receptores presentes no adipócito, impedindo assim tratamentos direcionados e eficazes para a obesidade (Dias, Paredes and Ribeiro 2018). Como qualquer outro tecido, está sujeito às ações do envelhecimento, o que reflete em mudanças significativas em sua abundância, distribuição, composição celular e sinalização endócrina, além de desempenhar um papel central no desenvolvimento da resistência à insulina, disfunção metabólica e inflamação, contribuindo para a disfunção adiposa relacionada à idade (Palmer and Kirkland 2016, Tchkonia et al. 2010). Entretanto, há lacunas na literatura considerando como foco o papel metabólico do tecido adiposo.

Durante os períodos de privação de energia, os adipócitos passam por uma maior taxa líquida de lipólise, isto é, a quantidade de lipídios que será queimada, que pode ser definida como a hidrólise do triacilglicerol em glicerol e ácidos graxos livres (ou ácidos graxos não-esterificados), que são liberados na vasculatura para uso por outros órgãos como substratos energéticos (Duncan et al. 2007, Fruhbeck et al. 2014, Rydén and Arner 2017). Em tempos de abundância, a insulina suprime a lipólise indiretamente como resultado do aumento do fluxo de glicose mediado pelo transportador de glicose tipo 4 (GLUT-4) por meio da glicólise, a via metabólica que converte a glicose em piruvato (Rutkowski, Stern and Scherer 2015). No entanto, uma parte deste piruvato é metabolizada em lactato, que sai do adipócito e medeia a inibição da lipólise dependente de insulina por meio de uma via autócrina do lactato (Rutkowski et al. 2015, Rydén and Arner 2017).

As catecolaminas são os principais agentes lipolíticos e glicolíticos (Langin 2006, Rydén and Arner 2017). No entanto, AII em maior quantidade, estimulada ou não por catecolaminas, é o mediador endógeno responsável por muitos efeitos cardiovasculares adversos, como hipertensão essencial, disfunção endotelial e fibrose (Borghi et al. 2016). Estudos em animais e

humanos sugerem que a AII pode modular a lipólise, porém esses resultados ainda são controversos na literatura. Boschmann et al. observaram que a infusão de AII por microdiálise inibe a lipólise no tecido adiposo de homens e mulheres eutróficos (Boschmann et al. 2001, Boschmann et al. 2002). No entanto, nos estudos posteriores, essa informação não foi confirmada, mostrando que a AII estimulou a lipólise em vez de inibi-la em homens eutróficos e obesos (Boschmann et al. 2003, Boschmann et al. 2006a). Em contraste com este cenário, Goossens trabalhou com a mesma técnica em adipócitos isolados de humanos eutróficos e obesos e encontrou atividade antilipolítica da AII, que seria justificada pela ação dos receptores AT1 (Goossens et al. 2004, Goossens et al. 2007). Esses estudos colocam a lipólise como um ponto final celular, não validando como a via realmente se comporta.

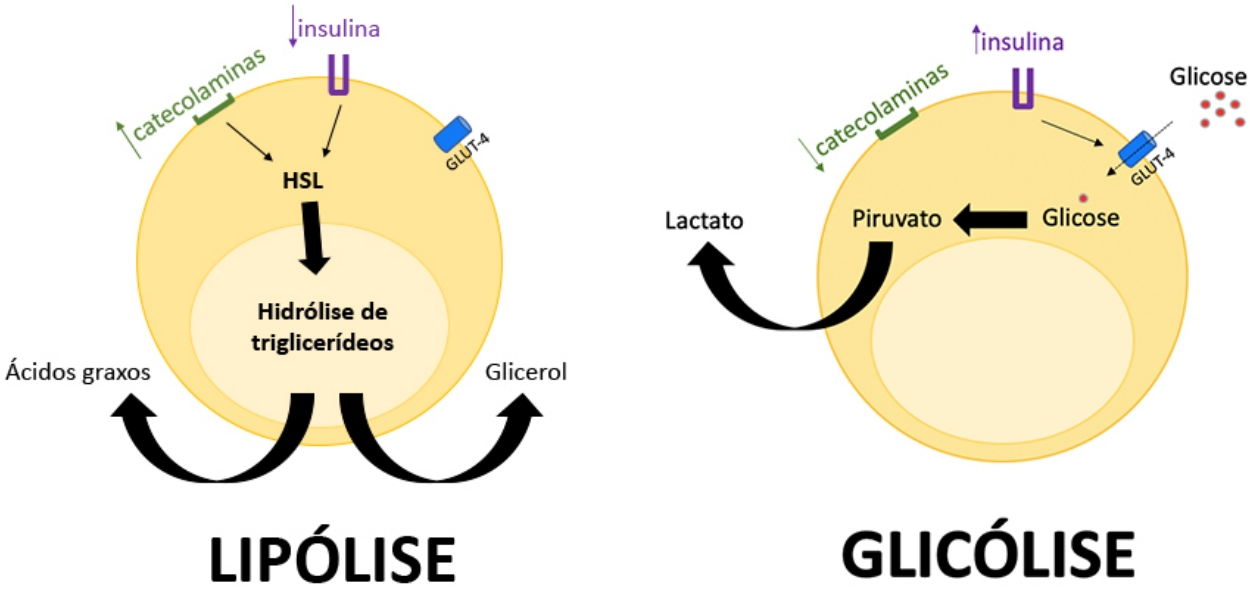

Figura 4 – Lipólise e glicólise em adipócitos

Os adipócitos participam ativamente na metabolização da glicose, sendo o grande responsável pela conversão dela em lactato, estimulado por insulina e catecolaminas (DiGirolamo, Newby and Lovejoy 1992, Faintrenie and Geloen 1996, Langin 2010). Fatores como tamanho do adipócito, sensibilidade a insulina e índice de obesidade do indivíduo são fatores que influenciam diretamente esta

taxa de conversão (Crandall et al. 1983, Lovejoy et al. 1992, Ahmed et al. 2010). Está bem estabelecido que as concentrações de lactato no sangue aumentam transitoriamente durante o período pós-prandial e durante o exercício agudo em indivíduos não diabéticos e diabéticos, com picos durante atividade física de alta intensidade (Heden, Liu and Kanaley 2017). Curiosamente, indivíduos obesos exibiram um nível elevado de lactato plasmático durante o jejum, que é revertido quando o indivíduo perde peso (Jansson et al. 1994, Lovejoy et al. 1992, DiGirolamo et al. 1992). Em ratos, altas concentrações de glicose no sangue foram acompanhadas pela maior liberação de lactato e menos ácidos graxos pelo tecido adiposo (Ahmed et al. 2010).

Alguns trabalhos apontam que as concentrações basais de lactato estão relacionados à resistência à insulina, com aumento da produção de lactato diretamente ligada à diminuição da sensibilidade à insulina causada pela destruição de receptores de insulina devido à hiperlactatemia (Consoli, Nurjhan and Gerich 1989, Lovejoy, Mellen and Digirolamo 1990, Chen, Varasteh and Reaven 1993). Entretanto, pacientes diabéticos tipo II apresentam quadro de hiperlactatemia em condições de jejum e diminuição das concentrações de lactato após a alimentação (DiGirolamo et al. 1992, Chen et al. 1993). Esse fato é intrigante, pois a carga oral de glicose dos alimentos pode refletir a resistência à insulina, diminuindo a capacidade da insulina de estimular a utilização da glicose e o metabolismo do lactato (DiGirolamo et al. 1992). Entretanto, o que não pode ser desconsiderado é que a resistência à insulina bem como a obesidade são acompanhadas de hiperativação do sistema nervoso simpático (Scott, Melhorn and Sakai 2012, Rafacho et al. 2013).

Objetivos Gerais

Caracterizar o perfil metabólico do tecido adiposo em ratos espontaneamente hipertensos (SHR), Wistar-Kyoto (WKY) e Wistar (WIS) e o seu envolvimento com o sistema renina-angiotensina-aldosterona.

Objetivos Específicos

- Investigar a relação morfométrica do adipócito e seu envolvimento na lactatemia em condição de jejum de adipócitos isolados de ratos normotensos e hipertensos;

- Investigar a produção de glicerol e lactato em adipócitos isolados de WIS, WKY e SHR com angiotensina II e noradrenalina, co-incubados ou não, na ausência ou presença de antagonistas seletivos dos receptores AT1 e AT2;

- Investigar e descrever as diferenças metabólicas ao longo do tempo em tecido adiposo branco e marrom de ratos normotensos e hipertensos utilizando a relação redox pela técnica de FLIM (Fluorescence Lifetime Imaging) antes e depois da instalação da hipertensão;

- Avaliar e descrever a presença dos fenômenos de browning e whitening em diferentes panículos adiposos brancos e marrom pela quantificação da proteína UCP-1 por Western Blot e de hormônios alvos do eixo Hipotálamo-Pituitária-Adrenal (HPA), Hipófise-Hipotálamo-Tireoide (HHP) e adipocinas antes e depois da instalação da hipertensão.

Para a apresentação desta tese, a metodologia e os resultados foram demonstrados nos manuscritos e/ou artigos gerados durante o seu desenvolvimento, seguindo as Normas para elaboração de teses/dissertações CCPG/001/2015 Art. 2º.

ARTIGOS E MANUSCRITOS

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A new perspective of lactatogenesis by isolated adipocytes

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ABSTRACT

Increased adipose tissue mass exhibited greater capacity of glucose transformation in lactate, highlighting lactatogenesis as a crucial factor in body size. Classically, lactate produced by isolated adipocytes are expressed per million of cells and were never correlated with their size. Spontaneously hypertensive rats (SHR) have a lower body weight and smaller adipocytes when compared to Wistar-Kyoto. We evaluated basal lactate by epididymal 15 weeks-old isolated adipocytes of SHR, Wistar-Kyoto and Wistar. Basal lactate was similar when expressed by one million cells. However, SHR adipocytes were smaller, so we adjusted the results by cell volume and SHR showed higher basal lactate production which was directly endorsed by hyperlactatemia in the presented conditions. Thereby, we suggest a new perspective on lactatogenesis analysis by adipocytes, which could be linked to the receptors density and associate enzymes. Moreover, we showed that the thin and hypertensive rats can be hyperlactemic in fasting conditions.

1. Introduction

Lactate is an important energy currency and is produced by different cells and under different conditions. It is well established that blood lactate concentrations increase transiently during the postprandial period and during acute exercise in nondiabetic and diabetic individuals and that greater increases occur with higher exercise intensities (Heden et al., 2017). Interestingly, obese subjects exhibited an elevated plasma lactate level during fasting, which is reversed when the subject loses weight (Jansson et al., 1994; Lovejoy et al., 1992; DiGirolamo et al., 1992). Thereby, the muscle cell selfishly produces lactate for its own consumption during the exercise and the extrahepatic tissues, as fat cells, distribute the energy to the whole body during acute energy deprivation.

The lactate production is stimulated by anaerobic glycolysis during muscle contraction and by glucose conversion to lactate in extrahepatic tissues to contribute to the gluconeogenesis in the liver (DiGirolamo et al., 1992). Lactate levels fluctuate particularly in relation to meals, however muscle does not contribute to the appreciable rise in circulating lactate levels after a meal (DiGirolamo et al., 1992). The lack of significant net lactate production by the muscle highlighted the adipose tissue as an active producer of lactate.

The adipose tissue has many functions linked to metabolism, such as thermogenesis, inflammation, production and secretion of hormones and substances associated with maintenance of the body's metabolic and functional balance (Gimeno and Klaman, 2005; Jing et al., 2013). Depending on the anatomical position in the body, adipose tissue contributes differently due to different mitochondrial density (Bjorndal et al., 2011). The epididymal fat pad is one of the largest adipose depots in rodents and have more mitochondria than other panicles, contributing more actively than the other fat pads (Deveaud et al., 2004; Bjorndal et al., 2011). The lactate production is extremely related to metabolic control and linked to blood glucose and epinephrine levels (DiGirolamo et al., 1992; Crandall et al., 1983; Faintrenie and Geloen, 1996). The adipocyte size, insulin sensitivity and the individual's obesity index are factors that affect the balance of the hypothalamic–pituitary–adrenal axis (Crandall et al., 1983; Lovejoy et al., 1992). Some works appoint that basal lactate levels are related to insulin resistance, with increased production of lactate directly linked to decreased insulin sensitivity caused by the destruction of insulin receptors due to hyperlactatemia (Consoli et al., 1989; Lovejoy et al., 1990; Chen et al., 1993). However, patients with diabetes type 2 presented an increase in lactate production under fasting conditions and a decrease in the lactate post-feed levels (DiGirolamo et al., 1992; Chen et al., 1993). This fact is intriguing because oral glucose load from food might reflect insulin resistance, decreasing the capacity for insulin to stimulate glucose

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utilization and metabolism to lactate (Lovejoy et al., 1992).

The literature has already described the relationship between lactate synthesis and size of adipocytes. Lactate production from glucose is increased in large adipocytes from obese animals or human and can reach up to 40%–60% of glucose metabolized (Newby et al., 1989; DiGirolamo et al., 1992; Crege et al., 2014; Li et al., 2016). It is clear that the obese subject exhibited increased adipose tissue mass, resulting in a considerable amount of lactate produced. However, these findings suggest that increased adipose tissue mass exhibited hyperplasia followed by hypertrophy without any characterization of adipocyte size.

Insulin resistance, obesity and hypertension are diseases which coexist and can be linked, independent or sequential in the same subject. An individual with inadequate habits such as sedentary lifestyle and a diet rich in carbohydrates and fats will initially exhibit insulin resistance followed by obesity and ultimately hypertension. All these diseases have in common the hyperfunction of sympathetic nervous system (Scott et al., 2012; Rafacho et al., 2013; Borghi et al., 2016). Particularly, the increase in sympathoadrenal activity of spontaneously hypertensive rats (SHR) takes place between 4 and 6 weeks of age, which is followed by an increase in blood pressure present in adulthood (Conceicao-Vertamatti et al., 2017; Judy et al., 1978). The importance of this model has been attributed to the similarity of its pathophysiology with essential hypertension in humans (Pravenec et al., 2014; Trippodo and Frohlich, 1981). This model also exhibits overactivity in the thyroid axis, exhibiting increased rate of metabolic activity that reflect in hypolipodistrophy and lean phenotype since birth (Garcia et al., 1995; Conceicao-Vertamatti et al., 2017). Previous study of our group confirmed that SHR exhibited hypolipodistrophy due to smaller adipocytes when compared to WKY and WIS (Gouvea et al., 2016; Morais et al., 2017b). The Wistar-Kyoto rat (WKY), inbred from the SHR strain as a control, does not develop hypertension but exhibit the same SHR high catecholamines pattern (Conceicao-Vertamatti et al., 2017). However, the SHR presented lower body weight when compared to normotensive groups, such as WKY and Wistar (WIS) strains (Silva et al., 2017; Morais et al., 2017a). Within this context, we aimed in this work to evaluate and relate the basal lactate production in these strains to their morphometry and analyze the impact of this phenomenon in lactatemia, since these levels during fasting are linked to the lactate production by adipocytes.

2. Methods

2.1. Animals

Male 15-weeks-old WIS (HanUnib:WH), WKY (NTacUnib:WKY) and SHR (SHR/NTacUnib) rats (n = 5/6 per cohort) (*Rattus norvegicus*) divided into two cohorts (for lactate quantification and morphometric assays) were used for the experiments. All animals were provided by Multidisciplinary Center for Biological Research (CEMIB - UNICAMP). The rats were housed in collective cages (3 rats per cage) at 22 °C on a 12 h light-dark cycle (lights on at 06:30 a.m.) with *ad libitum* access to standard chow (Labina Purina®) and filtered water. The experimental protocols were approved by the Committee for Ethics in Animal Experimentation of the Institute of Biology (UNICAMP, 4073-1), in accordance with NIH guidelines. The assays always started around 9 a.m. in order to avoid any interference in the circadian rhythmicity, after overnight fasting (12–16 h). The rats were anaesthetized with tiletamine 29 mg kg^{-1} and zolazepam 29 mg kg^{-1}, i.p. (Zoletil 50® - Virbac Laboratories, Carros, France); and xylazine 12.88 mg kg^{-1}, i.p. (Anasedan® - Sespo Ind. e Com. Ltda, Paulínia/SP, Brazil). They were euthanized by anesthetic overdose.

2.2. Serum analyses

Five mL of blood samples were collected from anaesthetized rats by cardiac puncture. The serum was obtained by centrifugation after 2 h at room temperature and after centrifuged at 10,000 rpm, 15 min, 4 °C. Serum samples were placed in aliquots at different vials and frozen until quantification.

2.3. Body weight, morphometry analysis and fresh culture of isolated adipocytes

During the assay, we measured the body weight after fasting. The entire epididymal pad was removed and weighted after euthanasia. Then, the adipocytes from epididymal fat pads were isolated according to Rodbell et al. and Grassi-Kassisse et al. with adaptation (Rodbell, 1964; Grassi-Kassisse et al., 2003). Then, the adipose tissue was fragmented and digested with 1 mg/mL collagenase (type II, from *Clostridium histoliticum*) in polyethylene tubes with 6 mL of Krebs-Ringer bicarbonate buffer containing Hepes (25 mM), glucose (6 mM), and bovine albumin (3%, BSA fraction V fatty-acid free), pH 7.4 (KRBA), at 37 °C with shaking (60 cycles/min) during 45 min. The isolated fat cells were filtered through a nylon mesh and washed 3 times with 6 mL KRBA buffer (3% BSA). The final volume of cellular suspension was adjusted to 50 mL with KRBA buffer (3% BSA). A 100 µL aliquot of cellular suspension were adjusted with KRBA to a 10% suspension: 10 µL of this suspension were transferred to a Mallassez chamber for adipocytes counting and morphometry through light microscopy Carl Zeiss Axiolab re 10x objective magnification (Zeiss, Oberkochen, Germany). Then, we used the software IMAGE J (National Institute of Health, USA) to perform the morphometry.

Aliquots of the cell suspension (70,000 to 200,000 cells) were incubated with gentle shaking (60 cycles/min) in vials containing KRBA. After 60 min, the tubes were placed in an ice bath and the adipocytes were removed by aspiration. The infranatant samples were frozen and, in a different day, were used to measure lactate. Basal results were determined in tubes containing the cellular suspension without any agonists or antagonist.

2.4. Lactate measure

The lactate quantification was performed by kits from Labtest (Lagoa Santa, MG/Brazil) according manufacturer's instructions, with some adjustments as described by Crege et al., (2014). All the results were adjusted per million cells of 60 min in a basal condition and then adjusted to volume of adipocytes. The results are expressed in nmol/mm^3.60min and obtained by dividing the results expressed per million cells by volume. The serum samples were diluted 1:5 to fit in the kit protocol and were expressed as mmol/mL. The standard curve was prepared in KRBA buffer.

2.5. Statistical analysis

Data are presented as means ± SEM with the number of experiments (n) performed in triplicate or duplicate. The normality was evaluated by D'Agostino-Pearson and then we performed Student's t-test for parametric and Mann-Whitney for nonparametric data. Statistical analysis was performed using Graph Pad Prism version 7.00 for Windows (Graph Pad Software, San Diego, California, USA). The acceptance level of significance was set at $p < 0.05$.

3. Results

When we compared the morphometry data, SHR exhibited lower body weight and lower epididymal fat pad weight when compared to its control WKY (Fig. 1A–B). About the normotensive strains, the WKY was heavier and exhibited large epididymal fat pad when compared to WIS (Fig. 1A–B). WKY presented the highest ($p < 0.05$) percentage of adipose pad weight in relation to body weight and SHR the lowest one ($p < 0.05$) (WIS: 1.44 ± 0.095%; WKY: 1.982 ± 0.079%; SHR: 0.338 ± 0.109%).

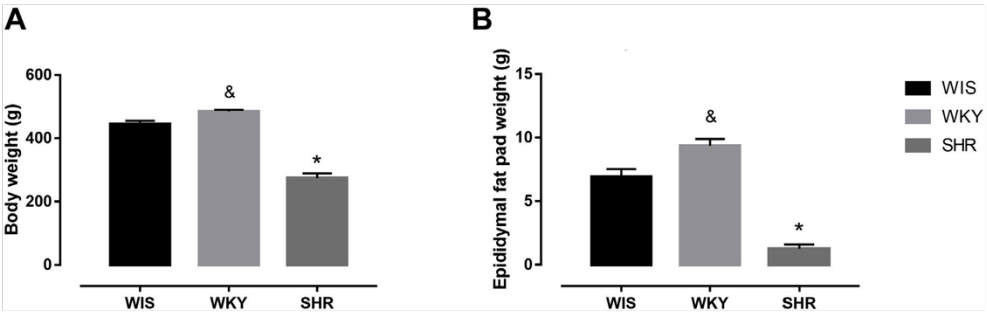

Fig. 1. Morphometric parameters of WIS, WKY and SHR rats. **A)** Body weight (g); **B)** Epididymal fat pad weight (g). Data are reported as the mean ± SEM (n = 6). *p < 0.05 SHR vs. WKY; &p < 0.05 WKY vs. WIS.

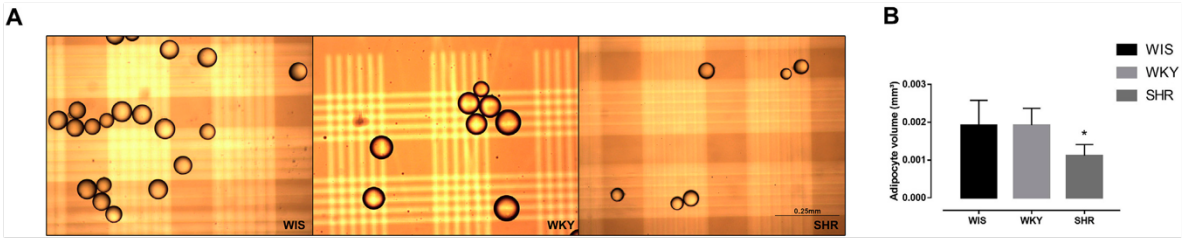

Fig. 2. Isolate adipocytes from WIS, WKY and SHR rats: **A)** in 10x objective magnification; **B)** Volume quantification. Data are reported as the mean ± SEM (n = 6). *p < 0.05 SHR vs. WKY.

At cellular level, we compared the volume of isolated adipocytes of epididymal adipose fat pad from these three strains (Fig. 2A). SHR exhibited lower volume ($p < 0.05$) (WKY: 0.0019 ± 0.0001 mm^3; SHR: 0.0011 ± 0.0001 mm^3) when compared to WKY. The WKY showed no differences in these parameters when compared to WIS, but exhibited a higher percentage of fat due to hyperplasia of adipocytes, not hypertrophy ($p < 0.05$; Fig. 2B).

When we analyzed the basal lactate production from isolated epididymal adipocytes, we observed that there were no differences between them when results are expressed as µmol.10^6cells.60 min (Fig. 3A). However, the results were intriguing because the smaller adipocytes produced the same lactate amount than the larger ones. Therefore, due to the important aspect related in the literature about the relationship between size and lactate production, we also investigate this parameter. This new point of view presented that SHR produced more lactate by volume when compared to WKY (Fig. 3B). The relevance of this phenomenon was endorsed by higher serum lactate in SHR, indicating that hypertensive and hypolipodystrophic rats were hyperlactatemic under fasting conditions (Fig. 3C).

4. Discussion

The role of lactate as an energy currency under catabolic condition is out of question, mainly under anaerobic exercise condition. The importance and understanding of hyperlactatemia under fasting condition seems to be linked to adrenergic activation in this case. The lactate duty in this condition is to provide substrate for the liver to maintain stable glucose levels. In all these situations, the adrenergic factor appears to play a prominent role, since the activation of alpha-adrenergic receptors matched by the increase in muscle glucose uptake, maintaining blood glucose levels relatively constant (Clark et al., 1983). The paradox is the effect of insulin, an anabolic hormone, which in addition to increasing glucose uptake in adipocytes, also induces the catabolism of glucose to lactate, even under no anaerobic condition. This effect remains obscure in lactatogenesis scientific world.

What we really know about lactate production is that the literature data point that adipocytes size directly influences the amount of glucose converted to this metabolite. In the proposed relationship, increased adipose tissue mass is able to produce more lactate (Newby et al., 1989; DiGirolamo et al., 1992; Crege et al., 2014; Li et al., 2016). However, during all these years, all works presented the results for lactate under

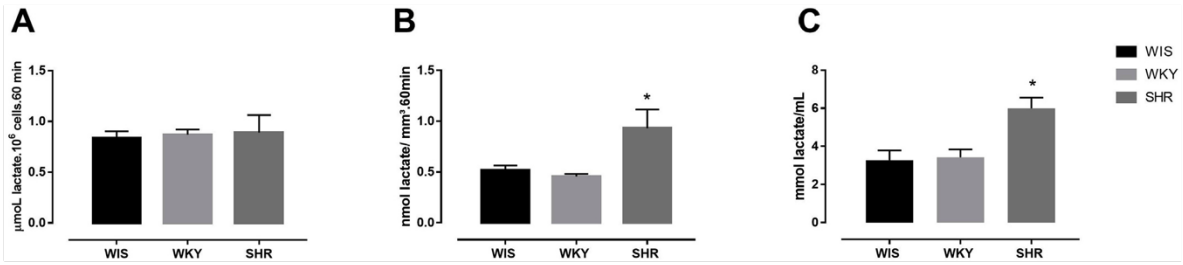

Fig. 3. Basal lactate production of epididymal isolated adipocytes from WIS, WKY and SHR rats. **A)** Expressed under cell number; **B)** Expressed by volume cell; **C)** Serum lactate levels. Data are reported as the mean ± SEM (n = 6). *p < 0.05 SHR vs. WKY; &p < 0.05 WKY vs. WIS.

cell number, expressed as amount produced by 1 million cells. Until now, no study has suggested a correlation between adipocyte size and its lactate production.

When we considered to quantify the lactate production under the morphometric aspect, we propose a new point of view. In our investigation, the values expressed under cell number (10^6 cells) did not indicate any differences in lactate production between normotensive and hypertensive strains. However, the values adjusted by cell volume showed that adipocytes from SHR produced more lactate than WKY. The relevance of this phenomenon was reflected in higher circulating lactate levels by SHR, since the mainly source of lactate in fasting condition are adipocytes (DiGirolamo et al., 1992). This is consistent with the fact that SHR had an increased lactate dehydrogenase (LDH) activity when compared to WKY (Zhou et al., 2017; Ooshima, 1973).

The SHR and WKY exhibit an age-dependent increase of the glucose-4 transporter (GLUT-4) in the gastrocnemius muscle and heart, when compared to WIS (Mondon and Reaven, 1988; Katayama et al., 1997). Moreover, SHR also has a higher density of α2 renal receptors that are associated with hyperinsulinemia and have been related to increased lactate efflux and their hypertensive profile (Mondon and Reaven, 1988; Katayama et al., 1997; Lockette et al., 1996; Kopp et al., 2011). On the other hand, higher serum lactate levels by SHR may also be related to insulin resistance (Gaboury et al., 1991). Choi et al. examined rat skeletal muscle and showed that increased blood lactate levels induces insulin resistance by suppressing glycolysis and impairing insulin signaling (Choi et al., 2002). Our work showed that smaller isolated adipocytes from hypertensive rats are able to produce higher amounts of lactate under fasting conditions. This phenomenon is extremely intriguing and deserves attention. The clearance of lactate to glucose serum levels was not followed by hyperlactatemic condition, not causing hyperglycemia. This means that the clearance was maintained and not increased.

We also have known that the adipocytes convert a sizeable part of glucose to lactate and glycerol according to their size limitations and exposure to glucose ambiance (Rotondo et al., 2017). Within this environment, it may be that SHR adipocytes deposit less triacylglycerols and have glucose metabolism diverted to lactate. With this brief information, we have reached a deadlock: is the SHR adipocyte smaller because of its ability to convert more glucose into lactate or because this conversion forms less glycerol, turning it smaller?

5. Conclusion

The analysis of lactate production under cell volume presented different results from those classically expressed under cell number and even closer to the profile exhibited by serum lactate levels. Thereby, we suggest a new perspective on lactatogenesis analysis, due to the performance of adipocytes in convert lactate from glucose, which could be linked the density of receptors and associate enzymes, besides the adipocytes size.

CRediT authorship contribution statement

Filipy Borghi: Data curation, Formal analysis, Investigation, Project administration, Resources, Visualization, Writing - original draft, Writing - review & editing. **Camila L. Morais:** Data curation, Formal analysis, Investigation, Visualization, Writing - original draft. **Carolina Silva:** Investigation. **Priscila C. da Silva:** Investigation. **Larissa Y. Ishizu:** Investigation, Writing - original draft. **Gustavo T. Costa:** Investigation. **Dora M. Grassi-Kassisse:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Visualization, Writing - original draft, Writing - review & editing.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mce.2019.110560.

References

Bjorndal, B., Burri, L., Staalesen, V., Skorve, J., Berge, R.K., 2011. Different adipose depots: their role in the development of metabolic syndrome and mitochondrial response to hypolipidemic agents. J Obes 2011, 490650.
Borghi, F., Sevá-Pessôa, B., Grassi-Kassisse, D.M., 2016. The adipose tissue and the involvement of the renin-angiotensin-aldosterone system in cardiometabolic syndrome. Cell Tissue Res. 366, 543–548.
Chen, Y.D., Varasteh, B.B., Reaven, G.M., 1993. Plasma lactate concentration in obesity and type 2 diabetes. Diabete Metab. 19, 348–354.
Choi, C.S., Kim, Y.B., Lee, F.N., Zabolotny, J.M., Kahn, B.B., Youn, J.H., 2002. Lactate induces insulin resistance in skeletal muscle by suppressing glycolysis and impairing insulin signaling. Am. J. Physiol. Endocrinol. Metab. 283, E233–E240.
Clark, M.G., Patten, G.S., Filsell, O.H., Rattigan, S., 1983. Co-ordinated regulation of muscle glycolysis and hepatic glucose output in exercise by catecholamines acting via alpha-receptors. FEBS Lett. 158, 1–6.
Conceicao-Vertamatti, A.G., Borghi, F., Canova, F., Grassi-Kassisse, D.M., 2017. History of vascular reactivity models and their involvement in hypertension pathogenesis. VASA 1–9.
Consoli, A., Nurjhan, N., Gerich, J., 1989. Rates of appearance and disappearance of plasma lactate after oral glucose: implications for indirect-pathway hepatic glycogen repletion in man. Clin. Physiol. Biochem. 7, 70–78.
Crandall, D.L., Fried, S.K., Francendese, A.A., Nickel, M., DiGirolamo, M., 1983. Lactate release from isolated rat adipocytes: influence of cell size, glucose concentration, insulin and epinephrine. Horm. Metab. Res. 15, 326–329.
Crege, D.R. X.d.O., Silveira, H.J.V., Chaim, É.A., Pareja, J.C., Géloen, A., Grassi-Kassisse, D.M., 2014. Sex difference in lactate production by adipocytes from lean humans. Open J. Endocr. Metab. Dis. 4.
Deveaud, C., Beauvoit, B., Salin, B., Schaeffer, J., Rigoulet, M., 2004. Regional differences in oxidative capacity of rat white adipose tissue are linked to the mitochondrial content of mature adipocytes. Mol. Cell. Biochem. 267, 157–166.
DiGirolamo, M., Newby, F.D., Lovejoy, J., 1992. Lactate production in adipose tissue: a regulated function with extra-adipose implications. FASEB J. 6, 2405–2412.
Faintrenie, G., Geloen, A., 1996. Alpha-1 adrenergic regulation of lactate production by white adipocytes. J. Pharmacol. Exp. Ther. 277, 235–238.
Gaboury, C.L., Karanja, N., Holcomb, S.R., Torok, J., McCarron, D.A., 1991. Patterns of insulin secretion and responsiveness in Wistar-Kyoto and spontaneously hypertensive rats. Am. J. Hypertens. 4, 661–666.
Garcia, S.I., Dabsys, S.M., Martinez, V.N., Delorenzi, A., Santajuliana, D., Nahmod, V.E., Finkielman, S., Pirola, C.J., 1995. Thyrotropin-releasing hormone hyperactivity in the preoptic area of spontaneously hypertensive rats. Hypertension 26, 1105–1110.
Gimeno, R.E., Klaman, L.D., 2005. Adipose tissue as an active endocrine organ: recent advances. Curr. Opin. Pharmacol. 5, 122–128.
Gouvea, S.G., Costa, G.T., Ishizu, L.Y., Conceição-Vertamatti, A.G., Borghi, F., Grassi-Kassisse, D.M., 2016. Adiposity of isolated adipocytes from fed hypertensive rats with the same age. In: XXIV Congresso de Iniciação Científica da Unicamp. Galoa Ciencia.
Grassi-Kassisse, D.M., Wolf-Nunes, V., Miotto, A.M., Farias-Silva, E., Souza Brito, A.R., Nunes, D.S., Spadari-Bratfisch, R.C., 2003. Sensitivity to beta-adrenoceptor agonists of adipocytes from rats treated with an aqueous extract of Croton cajucara Benth. J. Pharm. Pharmacol. 55, 253–257.
Heden, T.D., Liu, Y., Kanaley, J.A., 2017. Exercise timing and blood lactate concentrations in individuals with type 2 diabetes. Appl. Physiol. Nutr. Metabol. 42, 732–737.
Jansson, P.A., Larsson, A., Smith, U., Lönnroth, P., 1994. Lactate release from the subcutaneous tissue in lean and obese men. J. Clin. Investig. 93, 240–246.
Jing, F., Mogi, M., Horiuchi, M., 2013. Role of renin-angiotensin-aldosterone system in adipose tissue dysfunction. Mol. Cell. Endocrinol. 378, 23–28.
Judy, W.V., Watanabe, A.M., Henry, D.P., Besch, H.R., Aprison, B., 1978. Effect of l-dopa on sympathetic nerve activity and blood pressure in the spontaneously hypertensive rat. Circ. Res. 43, 24–28.
Katayama, S., Inaba, M., Maruno, Y., Morita, T., Awata, T., Oka, Y., 1997. Glucose intolerance in spontaneously hypertensive and Wistar-Kyoto rats: enhanced gene expression and synthesis of skeletal muscle glucose transporter 4. Hypertens. Res. 20,

279–286.
Kopp, U.C., Cicha, M.Z., Smith, L.A., 2011. Impaired interaction between efferent and afferent renal nerve activity in SHR involves increased activation of alpha2-adrenoceptors. Hypertension 57, 640–647.
Li, Y., Periwal, V., Cushman, S.W., Stenkula, K.G., 2016. Adipose cell hypertrophy precedes the appearance of small adipocytes by 3 days in C57BL/6 mouse upon changing to a high fat diet. Adipocyte 5, 81–87.
Lockette, W., Kirkland, K., Farrow, S., 1996. Alpha 2-adrenergic agonists increase cellular lactate efflux. Hypertension 27, 1104–1107.
Lovejoy, J., Mellen, B., Digirolamo, M., 1990. Lactate generation following glucose ingestion: relation to obesity, carbohydrate tolerance and insulin sensitivity. Int. J. Obes. 14, 843–855.
Lovejoy, J., Newby, F.D., Gebhart, S.S., DiGirolamo, M., 1992. Insulin resistance in obesity is associated with elevated basal lactate levels and diminished lactate appearance following intravenous glucose and insulin. Metabolism 41, 22–27.
Mondon, C.E., Reaven, G.M., 1988. Evidence of abnormalities of insulin metabolism in rats with spontaneous hypertension. Metabolism 37, 303–305.
Morais, C.L., Borghi, F., Silva, C., da Silva, P.C., Grassi-Kassisse, D.M., 2017a. Peso corporal, ingesta hídrica e alimentar entre ratos normotensos: Wistar e Wistar-Kyoto. In: XIII Congresso Aberto Aos Estudantes de Biologia (CAEB).
Morais, C.L., Borghi, F., Silva, C., da Silva, P.C., Grassi-Kassisse, D.M., 2017b. Relation of morphometry and basal lactate production in primary culture of isolated adipocytes from normotensive and hypertensive rats. In: XXV Congresso de Iniciação Científica da Unicamp (PIBIC), (Campinas: Galoá Proceedings).
Newby, F.D., Bayo, F., Thacker, S.V., Sykes, M., DiGirolamo, M., 1989. Effects of streptozocin-induced diabetes on glucose metabolism and lactate release by isolated fat cells from young lean and older, moderately obese rats. Diabetes 38, 237–243.
Ooshima, A., 1973. Enzymological studies on arteries in spontaneously hypertensive rats. Jpn. Circ. J. 37, 497–508.
Pravenec, M., Křen, V., Landa, V., Mlejnek, P., Musilová, A., Šilhavý, J., Šimáková, M., Zídek, V., 2014. Recent progress in the genetics of spontaneously hypertensive rats. Physiol. Res. 63 (Suppl. 1), S1–S8.
Rafacho, A., Goncalves-Neto, L.M., Ferreira, F.B., Protzek, A.O., Boschero, A.C., Nunes, E.A., Zoccal, D.B., 2013. Glucose homoeostasis in rats exposed to acute intermittent hypoxia. Acta Physiol. 209, 77–89.
Rodbell, M., 1964. Metabolism of isolated fat cells. I. Effects of hormones on glucose metabolism and lipolysis. J. Biol. Chem. 239, 375–380.
Rotondo, F., Ho-Palma, A.C., Remesar, X., Fernández-López, J.A., Romero, M.d.M., Alemany, M., 2017. Glycerol is synthesized and secreted by adipocytes to dispose of excess glucose, via glycerogenesis and increased acyl-glycerol turnover. Sci. Rep. 7, 8983.
Scott, K.A., Melhorn, S.J., Sakai, R.R., 2012. Effects of chronic social stress on obesity. Curr Obes Rep 1, 16–25.
Silva, C., Borghi, F., Morais, C.L., da Silva, P.C., Grassi-Kassisse, D.M., 2017. Peso corporal, ingesta hídrica e alimentar: avaliação em rato espontaneamente hipertenso e seu controle, o Wistar-Kyoto. In: XIII Congresso Aberto Aos Estudantes de Biologia (CAEB).
Trippodo, N.C., Frohlich, E.D., 1981. Similarities of genetic (spontaneous) hypertension. Man and rat. Circ. Res. 48, 309–319.
Zhou, R.Y., Wang, J.J., You, Y., Sun, J.C., Song, Y.C., Yuan, H.X., Han, X.M., 2017. [Effect of baicalin on ATPase and LDH and its regulatory effect on the AC/cAMP/PKA signaling pathway in rats with attention deficit hyperactivity disorder]. Zhong Guo Dang Dai Er Ke Za Zhi 19, 576–582.

Lipolysis and glycolysis of isolated adipocytes induced by hypertensive agents: angiotensin II and noradrenaline

Running tittle: Angiotensin II and noradrenaline effects in adipocytes

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Abstract

Background: Catecholamines and angiotensin II (AII) are potent vasoconstrictive agents and responsible for the hypertension. In addition, they also have metabolic action. AII was described to be directly involved in the dynamic balance of the body, such as the energy metabolism regulation of adipose tissue and the catecholamines are the main lipolytic and glycolytic agents in adipose tissue, providing glycerol and lactate as energy metabolites, respectively. Considering the hypolipodistrophic profile of Spontaneously Hypertensive Rats (SHR), we aimed to evaluate the production of glycerol and lactate induced by hypertensive agents in epididymal isolated adipocytes from 15-weeks-old SHR, Wistar-Kyoto (WKY) and Wistar rats (WIS). Methods: Adipocytes cells suspension was incubated with agonists (AII and noradrenaline) in a presence or not of antagonist (Losartan Potassium 10^{-4}M, PD 123319 5.6 nM and co-incubation). AII (10^{-17}M to 10^{-6}M) was added to adipocytes and kept in 60 min incubation. Results: AII receptor blockade and adipocyte stimulation by AII do not interfere in lactate production, although further investigations are still needed to understand how RAAS acts on adipocyte metabolism. Noradrenaline stimulation and its combination with AII increased glycerol production in all strains analyzed. On the other hand, the same combination caused a decrease in lactate production in WKY and SHR. Conclusions: These results suggest a modulation and/or competition of the downstream cascade of both hormones to exchange glycolytic for lipolytic metabolism. However, even with these phenomena, the results demonstrate that the hypolipodistrophy exhibited by SHR adipocytes is not due to any disturbance in lipolytic or glycolytic pathways.

Keywords: adipocytes; glycerol; lactate; angiotensin; noradrenaline; SHR.

Introduction

Classically, the renin-angiotensin-aldosterone system (RAAS) plays a crucial role in the equilibrium of the cardiovascular system, mainly due to the action of angiotensin II (AII) (1). In higher amounts, AII is responsible for many adverse cardiovascular effects such as hypertension, endothelial dysfunction and fibrosis (1). The introduction of AII-receptor blockers for type I (AT1) and II (AT2) have contributed to the longevity of hypertensive patients and to the understanding of this system (1, 2).

AII was described to be directly involved in the dynamic balance of the body, such as the energy metabolism regulation of adipose tissue. However, the catecholamines are the main lipolytic and glycolytic agents in adipose tissue, providing glycerol and lactate as energy metabolites, respectively (3-5). During energy deprivation, adipocytes undergoes a shift toward greater net lipolysis rates, which is the hydrolysis of triacylglycerol into fatty acids and glycerol that are released for use by other organs as energy substrates (6, 7). In times of abundance, insulin suppresses lipolysis and the glucose flux increase through GLUT-4 mediated glycolysis, the metabolic pathway that converts glucose into pyruvate (8, 9). However, portion of this pyruvate is metabolized to lactate, that exits the adipocyte and mediates insulin-dependent inhibition of lipolysis through an autocrine lactate loop (8, 10).

The spontaneously hypertensive rats (SHR) develops hypertension at 4–6 weeks of age without any type of intervention that mimicry the essential human hypertension (11, 12). The hormonal background of SHR and its control, the Wistar-Kyoto (WKY), are quite particular. Both strains present higher tonus of hypothalamus adrenal axis, with consequent higher levels of stress hormones when compared with Wistar rats (WIS), but without difference between them. However, plasma AII levels are not elevated in SHR (13, 14), excluding the renin-angiotensin-aldosterone system as the cause of hypertension in this strain. The WKY does not develop hypertension but exhibit the same SHR high catecholamines pattern (11, 15, 16). Furthermore, SHR exhibited lower weight and small adipocytes when compared to normotensive strains, such as WIS and WKY (17). This same pattern is observed when the rats are fed with high fat diet, which suggests an energy balance deregulation in SHR (18). Some previous

studies in essential hypertensive humans and SHR demonstrate that one of the factors associated with this kind of hypertension is the thinness and dyslipidemia, which would justify such phenotype (19, 20). This characteristic is also reinforced by the SHR adipocyte profile, characterizing the hypolipodistrophic phenotype (11, 13, 21). In this context, we aimed to evaluate the production of glycerol and lactate in isolated adipocytes from SHR, WKY and WIS with AII and Noradrenaline (NOR), co-incubated or not, in the absence and presence of selective AT1 and AT2 receptor antagonists.

Methods

Animals

Studies were conducted using 15-week-old WIS (HanUnib:WH), WKY(NTacUnib:WKY) and SHR (SHR/NTacUnib) male rats (n=5/6) (Rattus norvegicus). All animals were provided by Multidisciplinary Center for Biological Research (CEMIB - UNICAMP). Animals were housed in collective cages at 22ºC on a 12h light-dark cycle (lights on at 06:30 a.m.) with ad libitum access to standard chow (Labina Purina®) and filtered water. All animal housing, care, and experimental procedures were approved by the Committee for Ethics in Animal Experimentation of the Institute of Biology (UNICAMP, 4073-1) in accordance with NIH guidelines. Avoiding variables related to environmental conditions, we performed data collection in two different semesters, with two different cohorts containing three rats of each strain per cage. Transparent cages were used and the strains were placed separated from each other to avoid behavioral influence by the mechanism of mirror neurons (22). All assays were performed after overnight fasting rats. The rats were anaesthetized with tiletamine 29 $mg.kg^{-1}$ and zolazepam 29 $mg.kg^{-1}$, i.p. (Zoletil 50® - Virbac Laboratories, Carros, France); and xylazine 12.88 $mg.kg^{-1}$, i.p. (Anasedan® - Sespo Ind. e Com. Ltda, Paulínia/SP, Brazil). They were euthanized by anesthetic overdose.

Serum analyzes

Five mL of blood samples were collected from anesthetized rats by cardiac puncture. We wait 20 min to perform cardiac punction to avoid stress effects of manipulation (23). Plasma serum was obtained by centrifugation after 2 h at room temperature (10,000 rpm, 15 min, 4ºC). Serum aliquots were frozen until

quantification. Serum lactate were measured through Labtest kits (Lagoa Santa, MG/Brazil), according manufacturer's instructions, with some adjustments as described by Crege et al., 2014 (24).

Adipose tissue images and morphometry

The epididymal adipose tissue were removed, washed and kept in PBS at 37°C for the qualitative cell registration of this tissue. The images were obtained in the National Institute of Science and Technology on Photonics Applied to Cell Biology (INFABIC) at the State University of Campinas, using equipment Zeiss LSM 780 NLO microscope (Carl Zeiss, Jena, Germany) using a 40x water immersion objective, 1.2 N.A. (Carl Zeiss, Oberkochen, Germany). The morphometry was performed as described by Borghi et al., 2019 (17).

Adipocyte isolation

Isolation of adipocytes was performed as described by Borghi et al. (17). 2-5 g of epididymal adipose tissue was fragmented and digested with 1 mg/mL collagenase (type II, from Clostridium histoliticum), in polyethylene tubes with 6 mL of Krebs-Ringer bicarbonate buffer (KRBA) containing Hepes (25 mM), glucose (6 mM), and bovine albumin (3%, BSA fraction V fatty-acid free), pH 7.4 (KRBA), at 37°C with shaking (60 cycles/min) during 45 min. The isolated fat cells were filtered through a nylon mesh and washed 3 times with 6 mL KRBA buffer (3% BSA). The final volume of cellular suspension was adjusted to 50 mL with KRBA buffer (3% BSA). 100 µL of cellular suspension were adjusted with KRBA to a 10% suspension: 10 µL of this suspension were transferred to a Mallassez chamber for adipocytes counting through light microscopy. 70,000 to 200,000 cells were incubated with gentle shaking (60 cycles/min) in vials containing KRBA following the pharmacological assay described below.

Pharmacological assay

Adipocytes cells suspensions were incubating with agonists in a presence or not of antagonist. Angiotensin II (10^{-17}M to 10^{-6}M) or Noradrenaline (10^{-10}M to 10^{-4}M) was added to adipocytes and kept in 60 min incubation. When co-incubated, Noradrenaline (10^{-10}M to 10^{-4}M) was performed in a fixed Angiotensin II 10^{-7}M. These assays were performed with pre-incubation of 45 min with or without antagonists. The antagonists used were: Losartan Potassium 10^{-4}M (AT1 receptor antagonist (25)) and PD 123319 5.6 nM (AT2 receptor antagonist (25))

for 60 min. A third protocol performed a co-incubation of AT1 and AT2 blockers, i.e. combination of Losartan and PD 123319.

At the end of pharmacological assay, total time 105 min, the tubes were placed in an ice bath and the adipocytes were removed by aspiration. The infranatant samples were frozen and, in a different day, were used to glycerol and lactate assays, as respectively indicator of lipolysis and glycolysis. Basal results were determined in tubes containing the cellular suspension without agonists (with or without antagonists). The experiments were performed in different days, with one rat per day, and each pharmacological point in triplicate when possible. In the same week were performed assays from epididymal isolated adipocytes of WIS, WKY and SHR rats.

Lipolysis investigation

Glycerol was investigated in infranatant. Lipolysis, basal and stimulates, induced by NOR (10^{-10}–10^{-4}M) and AII (10^{-11}–10^{-5}M) were evaluated in glycerol released by adipocytes. The glycerol quantification was performed by kits from Laborclin (Pinhais, PR/Brazil), according manufacturer's instructions, with some adjustments as described by Crege et al. (24). The assay curve was performed in KRBA solution. All the results were adjusted per million cells of 60 min of agonist stimulation and in a basal condition, without agonist and then adjusted to area of adipocytes as described by Borghi et al. (17). The results are expressed in nmol/mm^2.60min.

Glycolysis investigation

Lactate was investigated in infranatant. Basal and stimulated glycolysis was evaluated in lactate released by adipocytes. The lactate quantification was performed by kits from Labtest (Lagoa Santa, MG/Brazil), according manufacturer's instructions, with some adjustments as described by Crege et al. (24). The assay curve was performed in KRBA solution. All the results were adjusted per million cells of 60 min of agonist stimulation and in a basal condition, without agonist and then adjusted to area of adipocytes as described by as described by Borghi et al. (17). The results are expressed in nmol/mm^2.60min.

Statistical analysis

The values are presented as means ± SEM with the number of experiments (n) performed in triplicate or duplicate in different days. The

normality was confirmed by Shapiro-Wilk test and then we performed Student's t-test for parametric and Mann-Whitney for nonparametric data. All statistical analysis was done with GraphPad Prism version 8.00 (GraphPad Software, San Diego, California, USA). Differences were considered significant for $p<0.05$. EC_{50} values (half maximal effective concentration) are expressed as pD_2 (-log EC_{50}), R_{max} and R_{min} for glycolysis and lipolysis. These data were calculated by GraphPad Prism version 8.00 considering the responses induced by co-incubated agonists or not, in the presence and absence of the AT1 and/or AT2 blockers.

Results

Morphometry

The WKY adipocytes from epididymal fat pad were visually larger when compared to its control (WIS) and SHR, which exhibited smaller adipocytes when compared to its normotensive control (WKY) (Figure 1).

Basal conditions of isolated adipocytes

Basal lipolysis was evaluated by analyzing glycerol production by isolated adipocytes. There were no significantly differences between basal lipolytic responses of isolated adipocytes from the three different rat strains. A blockade in AT1 and AT2 (LOS+PD) activity in WIS and in AT2 (PD) activity in SHR inhibited the basal glycerol production when compared with the adipocyte without stimulus or blockers. Between groups, we observed a decrease in glycerol production when AT1 (LOS) activity was blockade in WKY when compared to its control WIS, and an increase on SHR response when compared with WKY (Figure 2A-C).

Basal glycolysis was evaluated by analyzing lactate production by isolated adipocytes. The basal lactate production was higher in isolated adipocytes from SHR rats and no differences were observed between WIS and WKY adipocytes. The basal lactate levels were not affected by the blockade of AT receptors. Nevertheless, WIS exhibits a non-significant increase in basal lactate production when AT1 (LOS) activity was blockade and WKY when AT2 (PD) and the co-incubation (LOS+PD) were used. On the other hand, SHR exhibited a mild non-significant decrease in basal lactate production in presence of blockers, isolated

or co-incubated. This result showed that the highest basal lactate production in this strain was nullified in the presence of antagonists (Figure 2D-F).

Angiotensin II-induced lipolysis and glycolysis

The AII-induced glycerol and lactate production in isolated adipocytes from WIS was not affected by AT1 and AT2 blockade (Figures 3A-F), however there was a significant decrease in the presence of the associated blockers at 10^{-8}M concentration (Figure 3C). The AII-induced glycerol and lactate production in isolate WKY adipocytes was also not affected by the AT1 and AT2 blockade, although the biphasic curve showed a non-significant upward vertical displacement in the presence of AT1 blocker (Figure 4A-C/E). The SHR AII-induced glycerol production in isolated adipocytes was significantly decreased by AT2 and AT1 + AT2 blockers at AII 10^{-7}M concentration (Figures 5 A-C). Lactate production was not significantly affected by the blockade of isolated or associated AT1 and AT2 receptors (Figure 5D-F).

The values of R_{min}, R_{max} and pD_2 values of all these upper data showed no significantly differences under AT blockers conditions (Table 1). The AUC for glycerol production stimulated by AII in WKY isolated adipocytes was significantly lower when compared with WIS isolated adipocytes, however the same stimulus for SHR isolated adipocytes induced higher AUC glycerol production when compared with WKY data. The AT blockers did not alter the response induced by AII in WKY isolated adipocytes, however attenuated the response in SHR isolated adipocytes (Table 2). No significantly differences where identified in AUC lactate values for AII stimulus in adipocytes from WIS, WKY or SHR rats (Table 2).

Comparison between different agonists and strains

Figure 6 illustrates lipolysis and glycolysis of adipocytes isolated from rats of different strains against stimuli from NOR or AII agonists isolated or co-incubated with fixed AII concentration at 10^{-7}M. Nor induced significant increase in glycerol production (full symbols) from 10^{-7}M for WIS, WKY and SHR (Figure 6 A/D/G). The isolated adipocytes from WKY rats exhibited lower glycerol values in presence of AII when compared to those from WIS or SHR, respectively (Figures 6B/E/H - full symbols). The lactate production was also not altered in this NOR concentration range and the presence of AII did not cause significant alterations in this phenomenon (Figures 6B/C/E/F/H/I – empty symbols). There

were no differences in amount of lactate production by isolated adipocytes when the stimulus was AII (Figures 6 A/D/G - empty symbols). This same behavior was verified in the combination of AII and NOR (Figures 6 C/F/ I).

There were no significant differences in Rmin and Rmax values for NOR-induced lipolysis or glycolysis alone or combined with AII 10^{-7}M (Table 1). The sensitivity of isolated adipocytes to different agonists was evaluated by pD_2 analysis, which demonstrated that lactate production by isolated SHR adipocytes were more sensitive to NOR. The AII and NOR co-incubation caused significant decrease in NOR sensitivity in WIS and SHR adipocytes for lactate production. The sensitivity for glycerol production was not significantly altered (Table 1). The analysis of these results in area under the curve (AUC) showed no significant differences (Table 2).

Discussion

Stress is the nonspecific response of the body to any demand upon it and its response is composed by two major axis: the hypothalamic-pituitary-adrenal (HPA) axis and sympathetic nervous system (SNS), that are triggered by stressor stimuli to restore allostasis (26, 27). The HPA axis is the major stress system in the body, which is involved in the production of the stress hormone cortisol by the adrenal glands, a glucocorticoid that affected the glucose and fat metabolism (28, 29). The SNS activation increases the circulating catecholamine concentrations, which are considered as master regulators of lipolysis, and stimulates renin release from juxtaglomerular cells, activating the renin-angiotensin-aldosterone system (RAAS) (30, 31), that is the most important regulator of renal and cardiovascular function and it plays a main role in the equilibrium of the cardiovascular system (1). However, this system can also interfere with adipocytes metabolism, which are considered important agents in body energy production, including participation in glycolytic balance, through lactate production (1, 5, 32).

The literature points that enlargement of fat cells may be one of the contributors to the increase of basal glycolysis (31). However, even with the larger adipocytes, WKY did not exhibit higher basal lactate production (5, 33). On the other hand, despite the smaller adipocytes, SHR presents higher basal

lactatogenesis, which may be associated with hyperinsulinemia, α_2 receptor density and/or increased activity and concentration of lactate dehydrogenase (LDH) (34-36). This phenomenon is validated by the hyperlactatemia presented by SHR as reported in Borghi et al. (17).

The expression of all mediators of the renin–angiotensin-aldosterone system in adipose tissue validates the lactate production by intrinsic stimulus in isolated adipocytes from our assays, although not significant, different responses were observed when AT receptor blockade was performed. The AT1 and AT2 have opposite effects in hypertension and mainly in the metabolism control, which AT2 activation lead an upregulation of adipose tissue lipogenesis and AT1 leads a downregulation of lipolysis (1, 37, 38). Both of these receptors are G-protein-coupled receptors and the activation triggers a number of intracellular signal transduction pathways, such as G-protein-mediated (G_q and G_i), JAK/STAT, and MAPK or ERK intracellular signaling cascades and many of the classical roles of the RAAS have been attributed to the activation of one or more of these signaling cascades(39). The ratio of AT1 to AT2 receptors was approximately 1 to 1 in WKY and 3 to 1 in SHR (40). This may also occur in adipose tissue and could justify possible differences in blockade sensitivity of these receptors.

In presence of agonist, we notice different responses in increasing concentrations of AII. The strains exhibit different behaviors, pointing to antagonistic functions for AII. The RAAS blockers in isolated fat cells did not demonstrate relevant effects on glycolytic activity.

Catecholamines are the main endogenous lipolytic agent and act by stimulating different adrenoceptor subtypes (β_{1-3} and $\alpha_{1,\ 2}$) to initiate the degradation of triacylglycerol into fatty acids and glycerol, that are linked to G-proteins (41). β-adrenoceptors coupled stimulatory G (G_s) proteins activate adenylate cyclase (AC), resulting in an increase in cyclic adenosine monophosphate (cAMP) (42). This activates the protein kinase A (PKA) complex, leading to the phosphorylation of hormone-sensitive lipase (HSL) and perilipin, a protein on the surface of lipid droplets in adipocytes (42). As a positive control, noradrenaline stimulated glycerol production in isolated adipocytes from all strains at 10^{-7}M, which corroborates with the literature (43).

The modulation of lipolysis by AII was not validated in this study, but the combination with norepinephrine showed increased lipolytic activity in all strains and also decreased glycolytic activity in WKY and SHR strains. There are controversies about what would be the action of AII on lipolytic modulation in the literature. Some authors have observed inhibitory action of AII on lipolysis in eutrophic humans and isolated adipocytes (44-46) while other studies have addressed lipolysis stimulation in obese and eutrophic individuals by AII (47, 48). Although its isolated action has not yet been verified, the combination with other catecholamines, such as norepinephrine, it may have a lipolytic effect.

Our results confirmed an improvement in the regulation of lactate production by NOR, which was observed in SHR, indicating an increase in production under this stimulation, as observed in SHR (3). However, increased glycerol and decreased lactate production on combined action of AII and NOR point to a shift from glycolytic to lipolytic metabolism. This phenomenon may be associated with the combination of NOR on adrenergic receptors or by AII blockade on AT1 receptors that are already associated with antilipolytic activity (1, 41). Another hypothesis would be that the lipolysis inhibition pathway by lactate could be compromised, since lactate concentrations are lower, which would also justify the increase of lipolysis in these conditions (49).

Conclusion

Angiotensin II receptor blockade and adipocyte stimulation by AII do not interfere in lactate production. Noradrenaline stimulation and its combination with AII increased glycerol production in all strains analyzed. On the other hand, the same combination caused a decrease in lactate production in WKY and SHR. These results suggest a modulation and/or competition of the downstream cascade of both hormones to exchange glycolytic for lipolytic metabolism. However, even with these phenomena, the results demonstrate that the hypolipodistrophy exhibited by SHR adipocytes is not due to any disturbance in lipolytic or glycolytic pathways.

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Conflict of interest

The authors declare no competing interests.

References

1. Borghi F, Sevá-Pessôa B, Grassi-Kassisse DM. The adipose tissue and the involvement of the renin-angiotensin-aldosterone system in cardiometabolic syndrome. Cell Tissue Res. 2016;366(3):543-8.

2. Briasoulis A, Bakris GL. The OSCAR for cardiovascular disease prevention in chronic kidney disease goes to blood pressure control. Kidney Int. 2013;83(1):20-2.

3. Faintrenie G, Géloën A. Alpha-1 adrenergic regulation of lactate production by white adipocytes. J Pharmacol Exp Ther. 1996;277(1):235-8.

4. Higareda-Almaraz JC, Karbiener M, Giroud M, Pauler FM, Gerhalter T, Herzig S, et al. Norepinephrine triggers an immediate-early regulatory network response in primary human white adipocytes. BMC Genomics. 2018;19(1):794.

5. DiGirolamo M, Newby FD, Lovejoy J. Lactate production in adipose tissue: a regulated function with extra-adipose implications. FASEB J. 1992;6(7):2405-12.

6. Duncan RE, Ahmadian M, Jaworski K, Sarkadi-Nagy E, Sul HS. Regulation of lipolysis in adipocytes. Annu Rev Nutr. 2007;27:79-101.

7. Rydén M, Arner P. Subcutaneous Adipocyte Lipolysis Contributes to Circulating Lipid Levels. Arterioscler Thromb Vasc Biol. 2017;37(9):1782-7.

8. Rutkowski JM, Stern JH, Scherer PE. The cell biology of fat expansion. J Cell Biol. 2015;208(5):501-12.

9. Yin C, Liu WH, Liu Y, Wang L, Xiao Y. PID1 Alters the Antilipolytic Action of Insulin and Increases Lipolysis via Inhibition of AKT/PKA Pathway Activation. PloS one. 2019;14(4).

10. Ahmed K, Tunaru S, Tang C, Müller M, Gille A, Sassmann A, et al. An autocrine lactate loop mediates insulin-dependent inhibition of lipolysis through GPR81. Cell Metab. 2017;11(4):311-9.

11. Conceicao-Vertamatti AG, Borghi F, Canova F, Grassi-Kassisse DM. History of vascular reactivity models and their involvement in hypertension pathogenesis. Vasa. 2017:1-9.

12. Zicha J, Kunes J. Ontogenetic aspects of hypertension development: analysis in the rat. Physiol Rev. 1999;79(4):1227-82.

13. Borghi F, Silva C, da Silva PC, Ferrucci DL, Morais CL, Conceição-Vertamatti AG, et al. The Influence of Hypertensive Environment on Adipose Tissue Remodeling Measured by Fluorescence Lifetime Imaging in Spontaneously Hypertensive Rats. Molecular and cellular endocrinology. 2020;506.

14. Campbell DJ, Duncan AM, Kladis A, Harrap SB. Angiotensin peptides in spontaneously hypertensive and normotensive Donryu rats. Hypertension. 1995;25(5):928-34.

15. Berg T. Altered β1-3-adrenoceptor influence on α2-adrenoceptor-mediated control of catecholamine release and vascular tension in hypertensive rats. Front Physiol. 2015;6:120.

16. Berg T. β3-adrenoceptors inhibit stimulated norepinephrine release in spontaneously hypertensive rats. Front Physiol. 2014;5:499.

17. Borghi F, Morais CL, Silva C, da Silva PC, Ishizu LY, Costa GT, et al. A new perspective of lactatogenesis by isolated adipocytes. Mol Cell Endocrinol. 2019;498:110560.

18. Furedi N, Miko A, Aubrecht B, Gaszner B, Feller D, Rostas I, et al. Regulatory Alterations of Energy Homeostasis in Spontaneously Hypertensive Rats (SHR). J Mol Neurosci. 2016;59(4):521-30.

19. Swislocki A, Tsuzuki A. Insulin resistance and hypertension: glucose intolerance, hyperinsulinemia, and elevated free fatty acids in the lean spontaneously hypertensive rat. Am J Med Sci. 1993;306(5):282-6.

20. Penesova A, Cizmarova E, Belan V, Blazicek P, Imrich R, Vlcek M, et al. Insulin resistance in young, lean male subjects with essential hypertension. J Hum Hypertens. 2011;25(6):391-400.

21. Costa GT, Ishizu LY, Conceição-Vertamatti AG, Grassi-Kassisse DM. Morphometry of isolated adipocytes from rats of two models of hypertension. XXIII Congresso de Iniciação Científica da Unicamp.2015.

22. Bonini L. The Extended Mirror Neuron Network: Anatomy, Origin, and Functions. Neuroscientist. 2016.

23. Lee G, Goosens KA. Sampling Blood from the Lateral Tail Vein of the Rat. J Vis Exp2015.

24. Crege DRXdO, Silveira HJV, Chaim ÉA, Pareja JC, Géloen A, Grassi-Kassisse DM. Sex Difference in Lactate Production by Adipocytes from Lean Humans. Open Journal of Endocrine and Metabolic Diseases2014.

25. Yvan-Charvet L, Even P, Lamande N, Ferre P, Quignard-Boulange A. Prevention of adipose tissue depletion during food deprivation in angiotensin type 2 receptor-deficient mice. Endocrinology. 2006;147(11):5078-86.

26. Selye H. Stress without distress. New American Library1974

27. McEwen BS, Gianaros PJ. Stress- and allostasis-induced brain plasticity. Annu Rev Med. 2011;62:431-45.

28. Frodl T, O'Keane V. How does the brain deal with cumulative stress? A review with focus on developmental stress, HPA axis function and hippocampal structure in humans. Neurobiol Dis. 2013;52:24-37.

29. de Kloet ER, Joëls M, Holsboer F. Stress and the brain: from adaptation to disease. Nat Rev Neurosci. 2005;6(6):463-75.

30. Aldehni F, Tang T, Madsen K, Plattner M, Schreiber A, Friis UG, et al. Stimulation of renin secretion by catecholamines is dependent on adenylyl cyclases 5 and 6. Hypertension. 2011;57(3):460-8.

31. Morigny P, Houssier M, Mouisel E, Langin D. Adipocyte lipolysis and insulin resistance. Biochimie. 2016;125:259-66.

32. Caminhotto ReO, Sertié RA, Andreotti S, Campaãa AB, Lima FB. Renin-angiotensin system blockers regulate the metabolism of isolated fat cells in vitro. Braz J Med Biol Res. 2016;49(8).

33. Rotondo F, Ho-Palma AC, Remesar X, Fernández-López JA, Romero MDM, Alemany M. Glycerol is synthesized and secreted by adipocytes to dispose of excess glucose, via glycerogenesis and increased acyl-glycerol turnover. Sci Rep. 2017;7(1):8983.

34. Kopp UC, Cicha MZ, Smith LA. Impaired interaction between efferent and afferent renal nerve activity in SHR involves increased activation of alpha2-adrenoceptors. Hypertension. 2011;57(3):640-7.

35. Hodgins DS, Frohlich ED. Cardiac adenylate cyclase, cyclic nucleotide phosphodiesterase and lactate dehydrogenase in normotensive and spontaneously hypertensive rats. Biochemical Pharmacology. 1978;27(8):1179-85.

36. Ooshima A. Enzymological studies on arteries in spontaneously hypertensive rats. Japanese Circulation Journal. 1973;37(5):497-508.

37. Yvan-Charvet L, Quignard-Boulangé A. Role of adipose tissue renin-angiotensin system in metabolic and inflammatory diseases associated with obesity. Kidney Int. 2011;79(2):162-8.

38. Proença AR, Sertié RA, Oliveira AC, Campaña AB, Caminhotto RO, Chimin P, et al. New concepts in white adipose tissue physiology. Braz J Med Biol Res. 2014;47(3):192-205.

39. Bruce E, de Kloet AD. The intricacies of the renin angiotensin system in metabolic regulation. Physiol Behav. 2017;178:157-65.

40. Andresen BT, Romero GG, Jackson EK. AT2 receptors attenuate AT1 receptor-induced phospholipase D activation in vascular smooth muscle cells. J Pharmacol Exp Ther. 2004;309(1):425-31.

41. Bartness TJ, Liu Y, Shrestha YB, Ryu V. Neural innervation of white adipose tissue and the control of lipolysis. Front Neuroendocrinol. 2014;35(4):473-93.

42. Jocken JW, Blaak EE. Catecholamine-induced Lipolysis in Adipose Tissue and Skeletal Muscle in Obesity. Physiology & behavior. 2008;94(2).

43. Qvisth V, Hagström-Toft E, Enoksson S, Moberg E, Arner P, Bolinder J. Human Skeletal Muscle Lipolysis Is More Responsive to Epinephrine Than to Norepinephrine Stimulation in Vivo. The Journal of clinical endocrinology and metabolism. 2006;91(2).

44. Boschmann M, Ringel J, Klaus S, Sharma AM. Metabolic and hemodynamic response of adipose tissue to angiotensin II. Obes Res. 2001;9(8):486-91.

45. Boschmann M, Jordan J, Schmidt S, Adams F, Luft FC, Klaus S. Gender-specific response to interstitial angiotensin II in human white adipose tissue. Horm Metab Res. 2002;34(11-12):726-30.

46. Goossens GH, Blaak EE, Arner P, Saris WH, van Baak MA. Angiotensin II: a hormone that affects lipid metabolism in adipose tissue. Int J Obes (Lond). 2007;31(2):382-4.

47. Boschmann M, Jordan J, Adams F, Christensen NJ, Tank J, Franke G, et al. Tissue-specific response to interstitial angiotensin II in humans. Hypertension. 2003;41(1):37-41.

48. Boschmann M, Adams F, Schaller K, Franke G, Sharma AM, Klaus S, et al. Hemodynamic and metabolic responses to interstitial angiotensin II in normal weight and obese men. J Hypertens. 2006;24(6):1165-71.

49. Ahmed K, Tunaru S, Tang C, Müller M, Gille A, Sassmann A, et al. An Autocrine Lactate Loop Mediates Insulin-Dependent Inhibition of Lipolysis through GPR81. Cell Metabolism. 2010;11(4):311-9.

Tables

Table 1 – Minimum response (R_{Min}), maximum response (R_{Max}) and pD_2 values of lactate and glycerol production under the stimulus of NOR, AII and NOR+AII for isolated adipocytes of WIS, WKY and SHR rats (nmol/mm^2.60min).

	Glycerol			Lactate		
	R_{min}	R_{max}	pD_2	R_{min}	R_{max}	pD_2
AII						
WIS	0.1136+0.01956	0.1958+0.04377	8.323+0.6355	0.1784±0.0203	0.2192±0.0341	-8.201±0.9014
WKY	0.0744+0.02468	0.1126+0.02977	8.091+0.5008	0.1816±0.0214	0.2804±0.0551	-9.173±0.6603
SHR	0.1489+0.03322	0.2119±0.05255	8.483±0.5543	0.2013±0.0247	0.2270±0.0281	-8.726±0.9669
NOR						
WIS	0.1278+0.2253	0.395+0.03569	6.599+0.1653	0.1649±0.0162	0.2063±0.0106	-6.547±0.6712
WKY	0.0999+0.01868	0.3657+0.05286	6.746+0.1417	0.1592±0.0409	0.3095±0.0547	-5.526±0.5918
SHR	0.1493+0.02097	0.5029+0.1265	7.013+0.4123	0.2035±0.0213	0.2837±0.0195	-9.203±0.8297*
NOR+AII						
WIS	0.1769±0.0509	0.3757±0.1177	7.412±0.384	0.0487±0.0799	0.1835±0.0219	-4.520±0.6382$^{\#}$
WKY	0.0858±0.0201	0.3217±0.1066	6.821±0.107	0.1957±0.0305	0.2362±0.0245	-6.839±0.9600
SHR	0.1184+0.0175	0.4445+0.102	7.042+0.133	0.0744±0.0828	0.2310±0.0217	-4.778±0.196$^{\#}$

Data are presented as mean ± SEM (n=4-6). *$p<0.05$ compared between strains in the same agonist; $^{\#}p<0.05$ compared to AII versus NOR+AII. Unpaired t test with Welch's correction and Mann Whitney test, when appropriated.

Table 2 - Area under curve (nmol/mm^2.60min) of lipolytic and glycolytic response of isolated adipocytes from epididymal fat pads of different strains rats.

AUC		WIS	WKY	SHR
Glycerol	AII	0.7411±0.1101	0.4761±0.0806*	0.8218±0.1033*
	AII+LOS	0.6012±0.1310	0.6409±0.1067	0.5098±0.0764$^{\#}$
	AII+PD	0.6363±0.1571	0.6072±0.1232	0.5408±0.0514$^{\#}$
	AII+LOS+PD	0.6550±0.1212	0.6530±0.1502	0.6295±0.0215$^{\#}$
	NE	1.6250±0.2135	1.2220±0.1739	1.7380±0.3341
	AII+NOR	1.6580±0.3658	1.2960±0.2594	1.6710±0.3415
Lactate	AII	0.3250±0.0352	0.3639±0.0264	0.4125±0.0561
	AII+LOS	0.2800±0.0354	0.3121±0.3783	0.3234±0.0399
	AII+PD	0.2651±0.0426	0.4596±0.0401	0.3680±0.0207
	AII+LOS+PD	0.2509±0.0517	0.3682±0.0383	0.3240±0.0442
	NE	0.2811±0.0518	0.4553±0.0632	0.3819±0.0646
	AII+NOR	0.3311±0.0504	0.5571±0.0933	0.4059±0.0555

Glycerol or lactate production in presence of angiotensin II (AII) alone or combined with AT blockers: Losartan 10^{-4}M (AT1; LOS), PD 123319 5.6nM (AT2; PD) and co-incubation (LOS+PD; AT1 and AT2) and Noradrenaline (NOR) alone or combined with AII (AII+NOR) for WIS, WKY and SHR. Adipocytes were incubated at 37°C and lipolysis was evaluated throughout glycerol and glycolysis throughout lactate released in the medium. Data are reported as the mean ± SEM (n=4-6). *$p<0.05$ compared between strains in the same agonist; $^{\#}p<0.05$ compared the same strain in the same agonist. Unpaired t test with Welch's correction and Mann Whitney test, when appropriated.

Figures

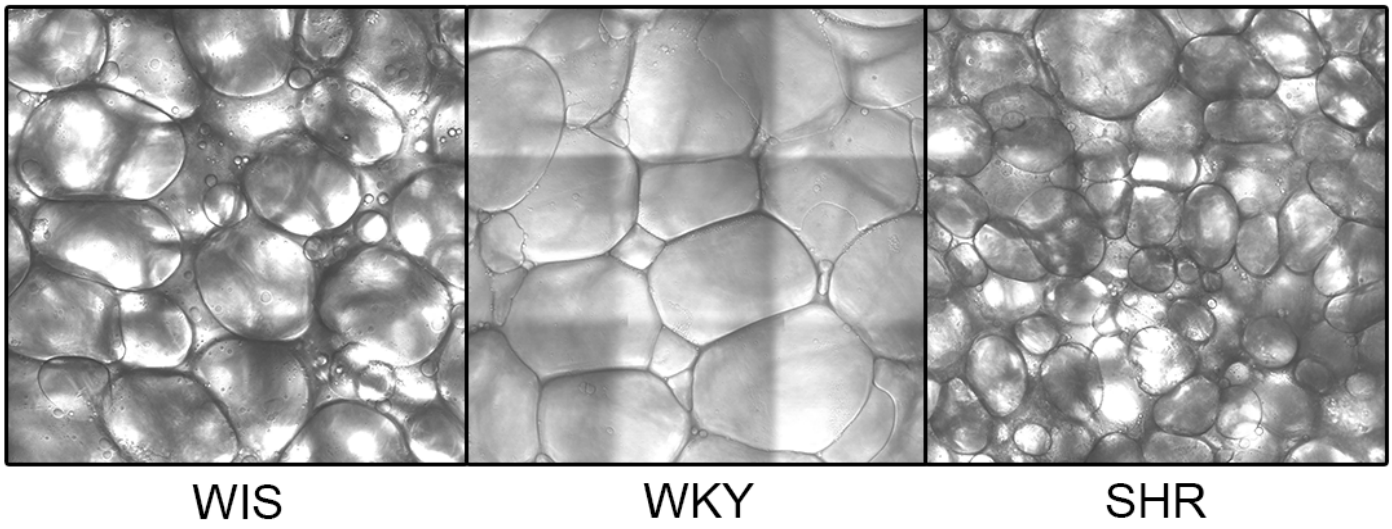

Figure 1 – Image of adipocytes from epididymal adipose tissue of WIS, WKY and SHR 15-week-old rats using a 40x water immersion objective.

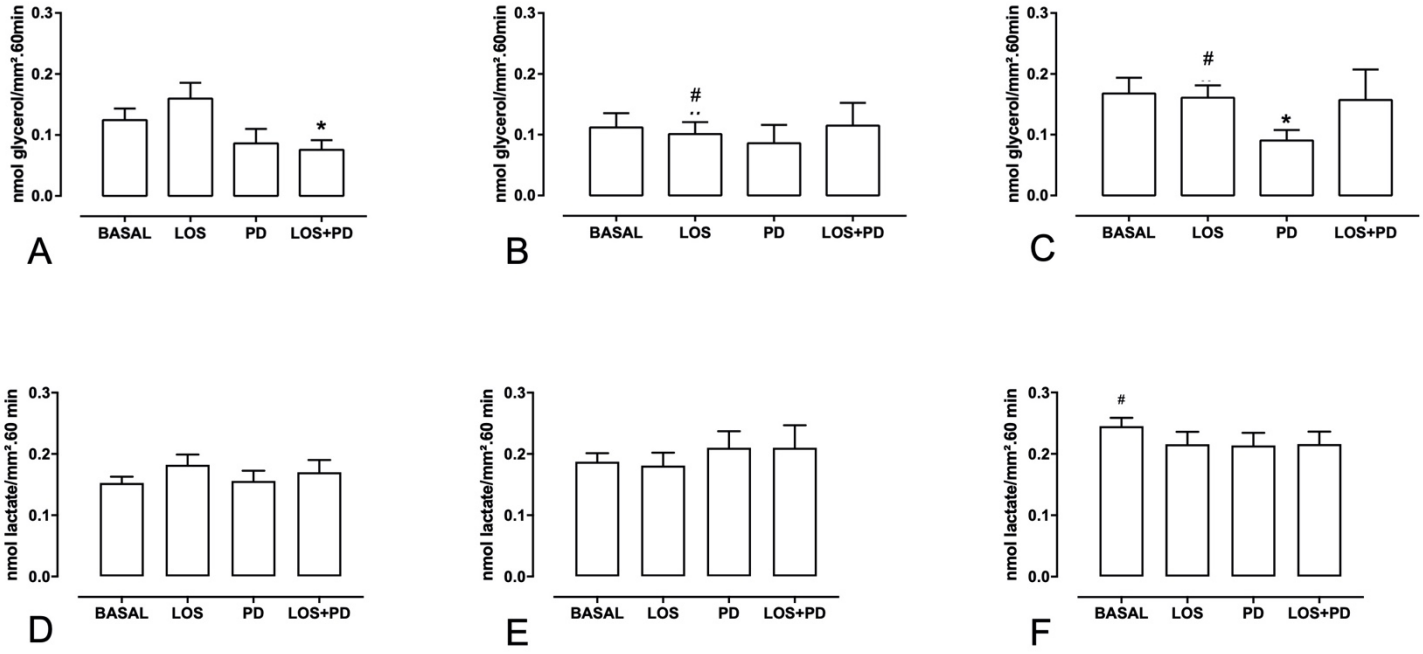

Figure 2 – Basal lipolytic and glycolytic response of isolated adipocytes from epididymal fat pads of different strains rats. Basal production of glycerol (BASAL) or with AT blockers: Losartan 10^{-4}M (AT1; LOS), PD 123319 5.6nM (AT2; PD) and co-incubation (LOS+PD; AT1 and AT2) for (A) WIS, (B) WKY and (C) SHR. Basal production of lactate (BASAL) or with AT blockers: Losartan 10^{-4}M (AT1; LOS), PD 123319 5.6nM (AT2; PD) and co-incubation (LOS+PD; AT1 and AT2) for (D) WIS, (E) WKY and (F) SHR. Adipocytes were incubated at 37°C and lipolysis was evaluated throughout glycerol and glycolysis throughout lactate released in the medium. Data are reported as the mean ± SEM (n=5-6). *p<0.05 basal vs AT blocker in the same strain; $^{\#}$p<0.05 for same blocker between strains.

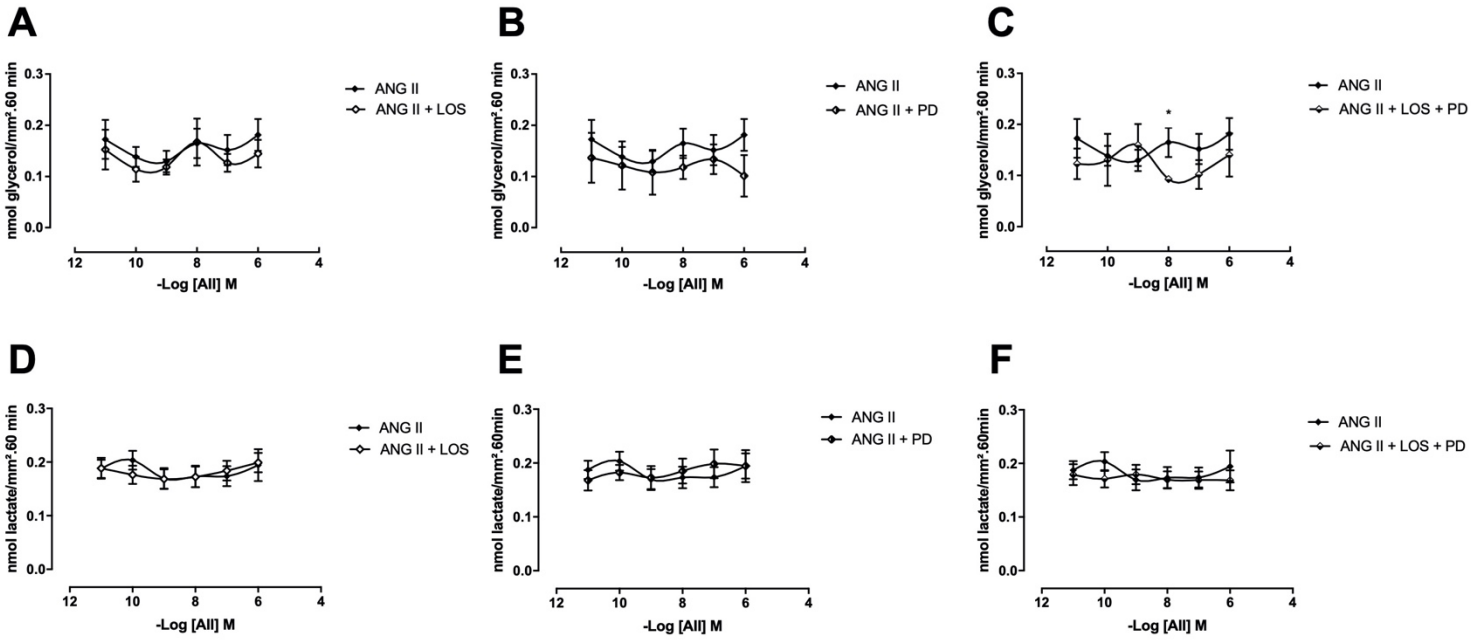

Figure 3 – WIS metabolic response of isolated epididymal fat cells incubated without (AII) or with AT blockers. Lipolytic response with: (A) Losartan 10-4M (AT1; LOS), (B) PD 123319 5,6nM (AT2; PD) and (C) co-incubation (LOS+PD; AT1 and AT2). Glycolytic response with: D) Losartan 10^{-4}M (AT1; LOS), (E) PD 123319 5,6nM (AT2; PD) and (F) co-incubation (LOS+PD; AT1 and AT2). Adipocytes were incubated at 37°C. Lipolysis was evaluated throughout glycerol and glycolysis throughout lactate released in the medium. Data are reported as the mean ± SEM (n=5-6). *p<0.05 blocker vs basal.

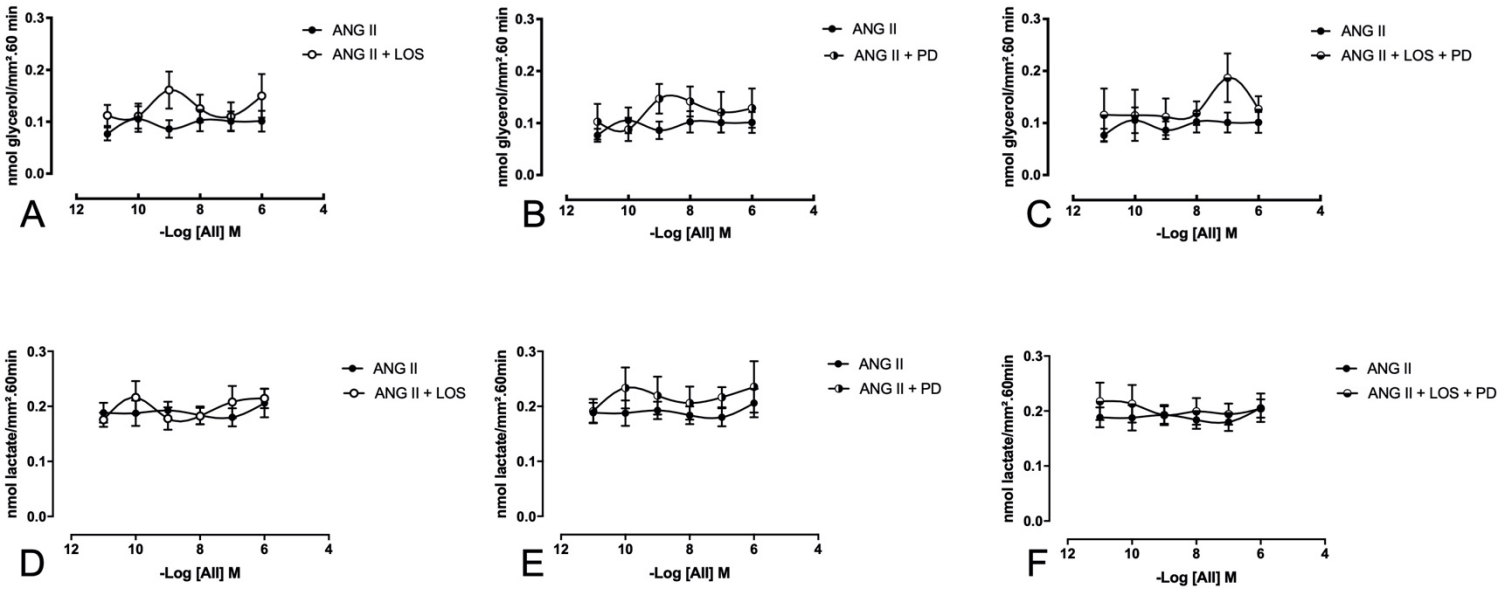

Figure 4 – WKY metabolic response of isolated epididymal fat cells incubated without (AII) or with AT blockers. Lipolytic response with: (A) Losartan 10^{-4}M (AT1; LOS), (B) PD 123319 5,6nM (AT2; PD) and (C) co-incubation (LOS+PD; AT1 and AT2). Glycolytic response with: D) Losartan 10^{-4}M (AT1; LOS), (E) PD 123319 5,6nM (AT2; PD) and (F) co-incubation (LOS+PD; AT1 and AT2). Adipocytes were incubated at 37°C. Lipolysis was evaluated throughout glycerol and glycolysis throughout lactate released in the medium. Data are reported as the mean ± SEM (n=5-6).

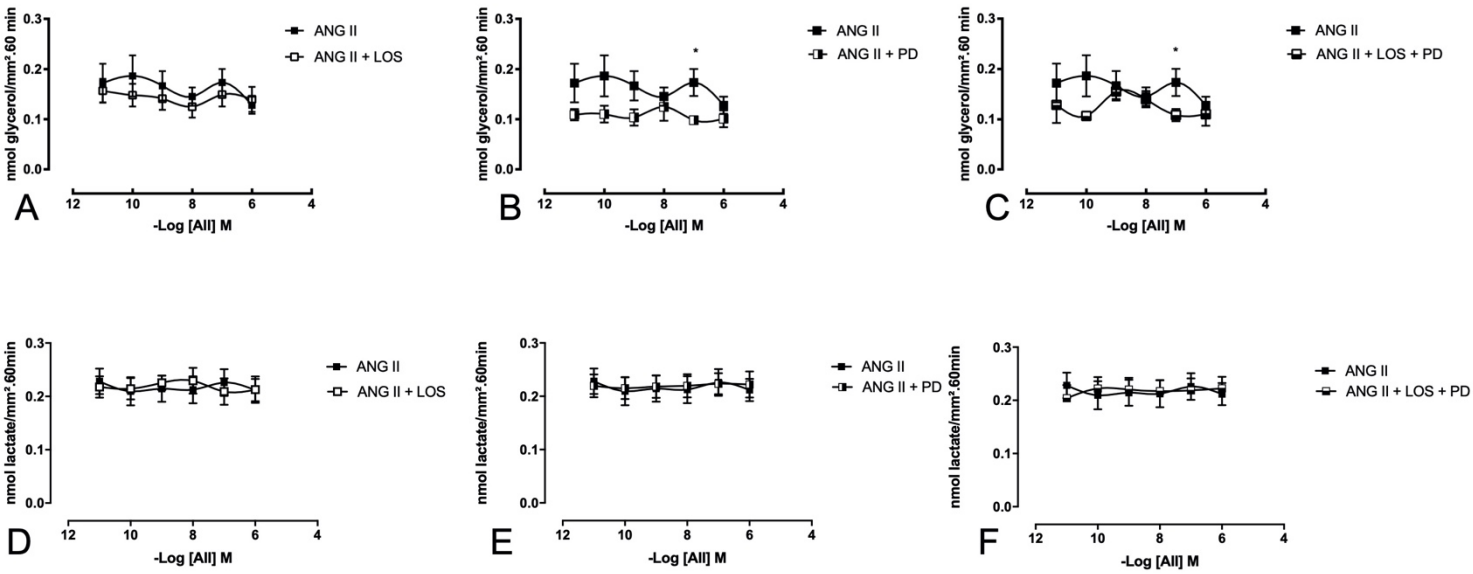

Figure 5 – SHR metabolic response of isolated epididymal fat cells incubated without (AII) or with AT blockers. Lipolytic response with: (A) Losartan 10^{-4}M (AT1; LOS), (B) PD 123319 5,6nM (AT2; PD) and (C) co-incubation (LOS+PD; AT1 and AT2). Glycolytic response with: D) Losartan 10-4M (AT1; LOS), (E) PD 123319 5,6nM (AT2; PD) and (F) co-incubation (LOS+PD; AT1 and AT2). Adipocytes were incubated at 37°C. Lipolysis was evaluated throughout glycerol and glycolysis throughout lactate released in the medium. Data are reported as the mean ± SEM (n=5-6). *p<0.05 blocker vs basal.

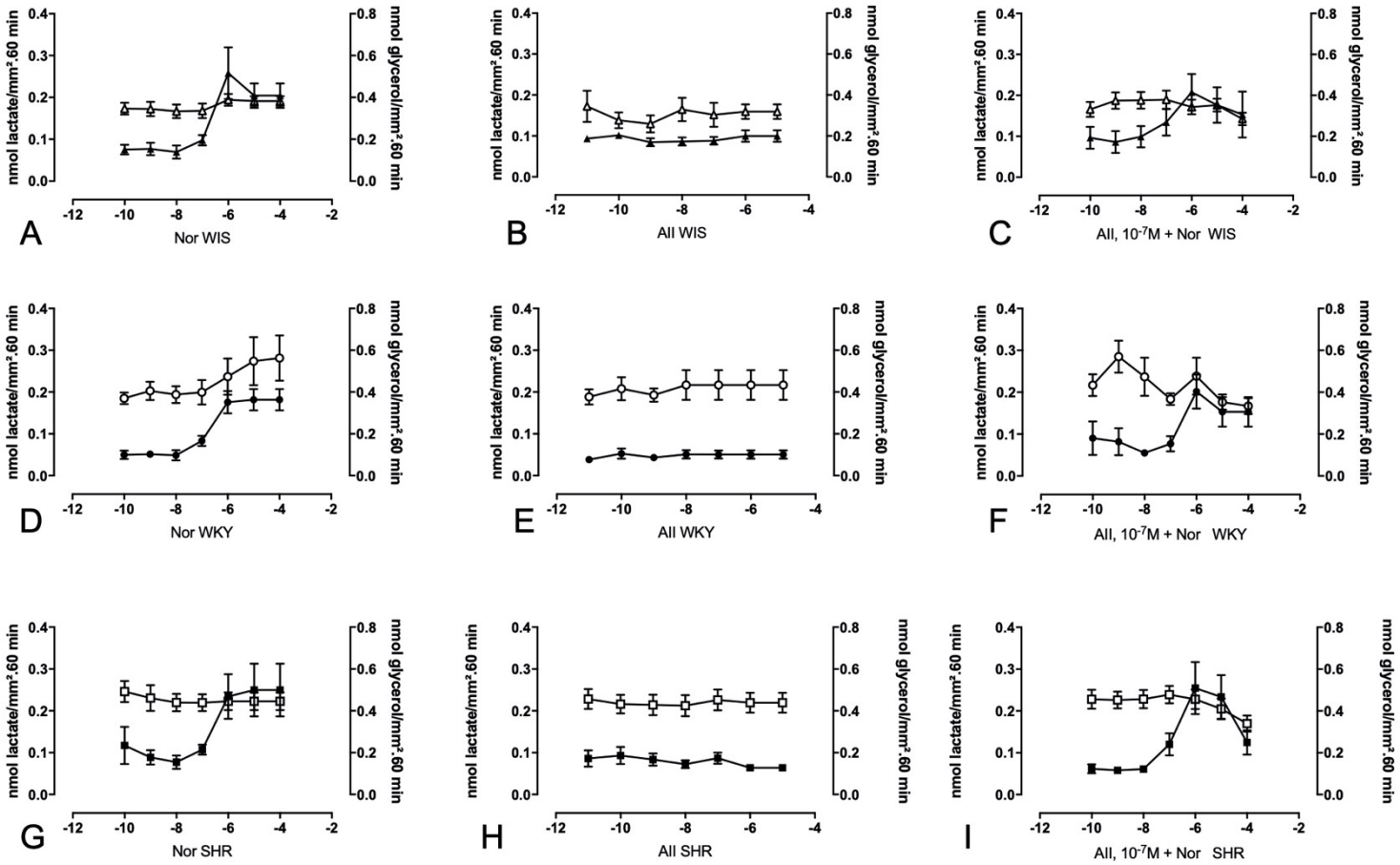

Figure 6 – Lipolytic and glycolytic response of isolated epididymal fat cells incubated with increasing concentrations of Angiotensin II (AII), Noradrenaline (NOR) and co-incubation AII, 10^{-7}M + NOR for: (A-C): WIS, (D-F): WKY and (G-I): SHR. Adipocytes were incubated at 37°C. Lipolysis was evaluated throughout glycerol released in the medium and glycolysis throughout lactate release in the medium. Data are reported as the mean ± SEM (n=5-6).

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The influence of hypertensive environment on adipose tissue remodeling measured by fluorescence lifetime imaging in spontaneously hypertensive rats

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ABSTRACT

There is a lack of information correlating low adiposity with hypertension experienced by Spontaneous Hypertensive Rats (SHR) or overweight and normotension in Wistar-Kyoto (WKY). We aimed to investigate this lipodystrophy phenomenon by measuring fluorescence lifetime (FLIM), optical redox ratio (ORR), serum levels of hypothalamic–pituitary–adrenal (HPA) and/or hypothalamic–pituitary–thyroid (HPT) hormones axes between Wistar, WKY and SHR before and after establishment of hypertension. Under high blood pressure, we evaluated serum adipokines. Brown adipose tissue was characterized as lower ORR and shorter FLIM compared to white adipose tissue. HPT axis showed a crucial role in the SHR adipose tissue configuration by attenuating whitening. The increased adiposity in WKY may act as a preventive agent for hypertension, since SHR, with low adiposity, establishes the disease. The hypertensive environment can highlight key adipokines that may result in new therapeutic approaches to the treatment of adiposity dysfunctions and hypertension.

1. Introduction

The correlation between hypertension and obesity is globally well recognized with large-scale population studies showing that 65–78% of hypertension cases are attributed to overweight (Das et al., 2018; Zhou and Qin, 2012). The adipose tissue (AT), an endocrine organ with active secretory activity (Scherer, 2006), is generally classified in three forms: (1) white adipose tissue (WAT), with major capacity for energy storage; (2) brown adipose tissue (BAT), which mediates non-shivering thermogenesis (Sanchez-Gurmaches and Guertin, 2014); and (3) brite/beige adipose tissue (Sanchez-Gurmaches and Guertin, 2014), that represents intermediate forms of white (white-like adipocytes in BAT) (Hill, 2015; Peirce et al., 2014) or brown (appearance of brown-like adipocytes in WAT) adipocytes (Peirce et al., 2014; Wang and Seale, 2016). Many endogenous and exogenous factors could effectively trigger the brite/beige cell recruitment such as cold, endocrine factors (sympathetic nervous system and thyroid activation), intermittent fasting or chemical compounds (Das et al., 2018; Montanari and Colitti, 2017; Brown, 2017).

Over its lifetime, the AT undergoes significant changes in abundance, distribution, cellular composition and endocrine signaling, being the only tissue with virtually unlimited growth potential at any stage of life (Pellegrinelli et al., 2016; Palmer and Kirkland, 2016). It can reduce its size in situations of malnutrition or during weight loss programs and show size recovery once diet is discontinued (MacLean et al., 2015). It is also known that the body fat distribution pattern is highly variable between individuals and some regional depots have favorable metabolic properties when compared to others (Sanchez-Gurmaches and Guertin, 2014; Lee et al., 2013; Ma et al., 2015).

The adipocyte is the main cellular component of the adipose tissue and reflects some particularities regarding its metabolic functions in different depots. The white unilocular adipocyte, characteristic of WAT, and the brown multilocular adipocyte found abundantly in the BAT, accumulate and store lipids. However, brown adipocytes are different

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from white adipocytes due to the presence of large amounts of mitochondrial uncoupling protein 1 (UCP-1), which decouples the oxidation of ATP substrate and release energy as heat (Peirce et al., 2014; Cannon and Nedergaard, 2004). Thermogenesis is mainly activated in response to cold due to the UCP-1 abundant expression triggered in response to adrenergic stimulation and thyroid hormones (Hernandez et al., 2011; Zheng et al., 2014; Wang et al., 2014). The active thyroid hormone, triiodothyronine (T3), amplifies the adrenergic stimulation of rat UCP-1 mRNA expression and contributes to the achievement of the maximal thermogenic capacity of BAT (Hernandez et al., 2011; Bianco et al., 1988). In addition to its thermogenic effects, once activated, BAT exhibits a large capacity of glucose and lipid uptake per gram of tissue, as demonstrated by its ability to normalize hyperglycemia and hyperlipidemia in mice models of hyperlipidemia and diabetes (Bartelt et al., 2011; Arbeeny et al., 1995). The cold-induced BAT activation increases energy expenditure, glucose uptake and improves weight control (Brown, 2017; Saito et al., 2009; Virtanen et al., 2009). Therefore, BAT and beige adipose tissue have become an attractive target for the treatment of obesity and related cardiometabolic diseases, including hypertension (Das et al., 2018).

Spontaneously hypertensive rats (SHR), a standard rat model of hypertension (Leong et al., 2015), were originated from inbred Wistar rats (WIS) and Wistar–Kyoto (WKY) non-hypertensive control rats (Okamoto and Aoki, 1963) and represent a powerful model for the study of the generalized lack of adipose tissue and altered body fat composition (Akinci et al., 2018) called hypolipodystrophy (Morais et al., 2017). The SHR model is characterized by increased sympathoadrenal activity that takes place between 4 and 6 weeks of age, which is followed by an increase in blood pressure in adulthood, fully established at 15 weeks of age (Judy et al., 1978; Kvetnansky et al., 1979). The hormonal background of SHR and its control WKY are quite particular. Both strains present higher tonus of hypothalamus adrenal axis, with consequent higher levels of stress hormones when compared with WIS, but without difference between them. However, plasma angiotensin II levels are not elevated in SHR (Campbell et al., 1995), excluding the renin-angiotensin-aldosterone system as the cause of hypertension in this strain. The WKY does not develop hypertension but exhibit the same high catecholamines pattern as SHR (Berg, 2014, 2015; Conceicao-Vertamatti et al., 2017). The SHR also exhibits elevated thyrotropin releasing hormone (TRH) activity associated with lower levels of leptin (Garcia et al., 1995; García et al., 2002) and high basal metabolism (Duntas and Brenta, 2012). Supporting this metabolic environment, the SHR exhibited lower body weight, smaller adipocytes and higher lactate production by adipocyte volume when compared to its control WKY (Borghi et al., 2019).

Among existing technologies for investigating metabolic activity, the cell redox ratio derived from the fluorescence intensity emitted by flavin adenine dinucleotide (FAD) and reduced nicotinamide adenine dinucleotide (NADH) uniquely enables a precise observation of changes in cellular metabolic rate and oxygen supply (Chance et al., 1979; Gulledge and Dewhirst, 1996; Ramanujam et al., 2001). This redox ratio has been shown to be a sensitive biomarker of changes associated with the relative rates of key metabolic and biosynthetic pathways in adipocytes, including glycolysis, oxidative phosphorylation and lipid synthesis (Alonzo et al., 2016; Huang et al., 2002). Optical methods based on fluorescence lifetime contrast such fluorescence lifetime imaging microscopy (FLIM) can detect biochemical and physiological transformations in tissue and are known to improve the specificity of fluorescence measurements (Fite et al., 2011). This method is intensity independent and measures the time that a molecule spends in an excited state before emission (Jones et al., 2018). In order to contribute to the cardiometabolic field and to suggest a relationship between the cause and/or the consequence of the hypolipodystrophy and hypertension environment experienced by SHR or the overweight and normotension in WKY, we aimed to describe metabolic differences in distinct adipose tissue depots before and after the established hypertension. We also investigated the involvement of the hypothalamic–pituitary–adrenal (HPA) and hypothalamic–pituitary–thyroid (HPT) axes.

2. Materials and methods

2.1. Animals

Studies were conducted using 6 and 15-week-old WIS (HanUnib:WH; n = 6), WKY (NTacUnib:WKY; n = 6) and SHR (SHR/NTacUnib; n = 6) male rats (*Rattus norvegicus*) weighed every week and before the experimental procedures. At least 6 animals of each age and group were used in each protocol. All animals were provided by the Multidisciplinary Center for Biological Research (CEMIB - UNICAMP). Animals were housed in collective cages (3 rats per cage) at 22 °C on a 12 h light-dark cycle (lights on at 06:30 a.m.) with *ad libitum* access to standard chow (Labina Purina®) and filtered water. Food and water intake were measured each three days by weighing what remained in the food cup and quantifying what remained in the water bottle and the values consumed were expressed by g/g or mL/g, respectively, of each animal in the cage. All animal housing, care, and experimental procedures were approved by the Committee for Ethics in Animal Experimentation (CEUA) of the Institute of Biology from Unicamp in Campinas, Brazil (no. 4073-1), in accordance with NIH guidelines. The rats were anesthetized with tiletamine 29 mg kg^{-1} and zolazepam 29 mg kg^{-1}, i.p. (Zoletil 50® - Virbac Laboratories, Carros, France); and xylazine 12.88 mg kg^{-1}, i.p. (Anasedan® - Sespo Ind. e Com. Ltda, Paulínia/SP, Brazil).

2.2. Blood pressure

Blood pressure was measured under anesthesia after 15 weeks, when hypertension has been estabilished (Conceicao-Vertamatti et al., 2017). Blood catheterization was performed with introduction of a cannula (PE 50) into the right carotid artery and connected to a pressure transducer strain-gauge type connected to a MLS370 amplifier/7 blood pressure Module (AD Instruments, Sydney, Australia). Data acquisition was performed by Power Lab 8/30 and results analysis were performed using LabChart Pro 7 (ADInstruments, Sydney, Australia). The mean arterial pressure was defined as: [systolic blood pressure + (2 X diastolic blood pressure)]/3.

2.3. Blood samples

Blood samples were collected from the tail vein of rats after overnight fasting (12–16h) and before anesthesia, at 9:00 a.m. to avoid anesthetic glycaemia effects. Blood glucose was evaluated using Roche Accu-Chek® Performa with strips (Cat No 06454011023, Lot No 474242), according to the manufacturer's instructions (Hoffmann–La Roche, Basel, Switzerland).

2.4. Serum analysis

Five mL of blood samples were collected from anesthetized rats by cardiac puncture. We waited 20 min to perform cardiac punction to avoid stress effects of manipulation (Lee and Goosens, 2015). Plasma serum was obtained by centrifugation of blood samples at 10,000 rpm, 15 min, 4 °C after 2 h at room temperature. Serum aliquots were frozen until quantification. Levels of thyroid-stimulating hormone (TSH), triiodothyronine (T3), corticosterone, adrenocorticotropic hormone (ACTH), leptin and adiponectin were measured using Milliplex Map Kit (Cat. #RSHMAG-69K; #RTHYMAG-30K; #RADPKMAG-80K; #RADPNMAG-81K-01; Millipore, Billerica, MA), following the manufacturer's recommendations. Adipokines were only measured after 15 weeks when significant differences in adiposity and food intake were observed.

Table 1
Body weight, food and water intake in WIS, WKY and SHR rats at 6 and 15 weeks of age.

	6th WEEK			15th WEEK		
	WIS	WKY	SHR	WIS	WKY	SHR
Body weight (g)	197.3 ± 4.2	221.3 ± 6.3$^{\#}$	123 ± 7.8$^{\#}$	467.7 ± 9.9*	526.8 ± 25.8*$^{\$}$	294.5 ± 9.9*$^{\$}$
Food intake (g/g of rat)	0.068 ± 0.019	0.107 ± 0.003	0.107 ± 0.007	0.058 ± 0.002	0.058 ± 0.004*	0.079 ± 0.003*$^{\$}$
Water intake (mL/g of rat)	0.148 ± 0.004	0.190 ± 0.0$^{\#}$	0.163 ± 0.007	0.090 ± 0.003*	0.097 ± 0.004*	0.139 ± 0.014$^{\$}$

Table 1 – Body weight before the procedure, food and water intake for WIS, WKY and SHR rats. Data are presented as mean ± SEM. *$p < 0.05$ compared between strains over the time; $^{\#}p < 0.05$ compared to control at 6th week (WIS vs. WKY; WKY vs. SHR); $^{\$}p < 0.05$ compared to control at 15th week (WIS vs. WKY; WKY vs. SHR); WIS = Wistar; WKY = Wistar-Kyoto; SHR = Spontaneously Hypertensive Rats.

2.5. Fluorescence lifetime imaging microscopy (FLIM)

Under anesthesia and after blood collection, the different adipose fat pads (inguinal white adipose pad – iWAT; and the interscapular BAT) were collected in accordance with the protocol proposed by Mann (Mann et al., 2014). Fat pads were washed and kept in PBS at 37 °C. Fluorescence lifetime imaging measurements (FLIM) from different adipose pads were examined in the National Institute of Science and Technology on Photonics Applied to Cell Biology (INFABIC) at the University of Campinas, using a Zeiss LSM 780 NLO microscope (Carl Zeiss, Jena, Germany) with a 40x water immersion objective, 1.2 N.A. (Carl Zeiss, Oberkochen, Germany). For the 2-Photon excitation laser source, Titanium: Sapphire MaiTai laser (Spectra-Physics, Mountain View, CA) was used with excitation at 740 nm. Image scan speed was set to 25.21 ms/pixel and image size was 256x256 pixels. Tissue sample was excited at 740 nm and emission filter employed was bandpass 405–590 nm. The data acquisition and processing were performed using SPCImage (Becker & Hickl GmbH, Berlin, Germany) (Adur et al., 2012; Pelegati et al., 2012).

2.6. Optical redox ratio calculation

To process the optical redox ratio, the fluorescence intensity of either NADH or FAD at every pixel was first taken as the total photon counts detected during the integration time without spatial binning. The optical redox ratio was acquired using fluorescence intensities for each pixel as FAD/(NADH + FAD) FLIM intensity. The mean redox ratio was used. Decreased redox ratio was used as a marker for increased cellular metabolic activity (Skala and Ramanujam, 2010; Skala et al., 2007; Digman et al., 2008).

2.7. Western Blotting

The adipose pads were separately pulverized in N_2 liquid and homogenized in cold RIPA lysis buffer (Merck Millipore, Billerica, MA, USA) containing protease inhibitor cocktail (PIC, Sigma-Aldrich) to obtain total protein extracts. Equal amounts of protein (BAT: 10 μg; iWAT: 50 μg) were used as total extracts, followed by SDS-PAGE (Mini-PROTEAN® TGX™ Precast Protein Gels, Bio-Rad) and Western Blot analysis with anti-UCP1 (D9D6X 1:1000; Cell Signaling) antibody. Anti-α tubulin (TU-02 1:1000; Santa Cruz Biotechnology) antibody was used as loading control. Immunocomplexes were detected using a luminol peroxidase chemiluminescence kit (Clarity Max™, Bio-Rad) and acquired using the Alliance 6.7, UVITECH imaging system. Protein band intensity was quantified using ImageJ software (National Institutes of Health, Bethesda, MD, USA).

2.8. Statistical analysis

Data are presented as means ± SEM. The normality was confirmed by D'Agostino & Pearson. Unpaired Student's t-test for parametric and Mann-Whitney for nonparametric data between controls (WIS vs. WKY; WKY vs. SHR) were performed using Graph Pad Prism 7 (Graph Pad Software, San Diego, USA). The acceptance level of significance was set at $p < 0.05$.

3. Results

3.1. Body weight, blood pressure, food and water intake

The SHR presented lower body weight than their control (WKY) and WKY rats presented higher body weight than their control (WIS) for all checked timepoints. At week 15, SHR exhibited higher mean arterial pressure (mmHg) when compared to WKY, which presented similar values to WIS (WIS: 103.8 ± 8.99; WKY: 103.7 ± 5.64; SHR: 122.9 ± 5.01*). WIS rats maintained the same food intake over time. WKY and SHR started with the same food intake at week 6, but a significant decrease was observed at week 15. WKY presented lower food intake than SHR animals at week 15, but exhibited eating behavior very similar to WIS. At week 6, WKY rats presented a higher water intake than WIS, but not statistically different from SHR. In addition, WIS and WKY animals presented reduced water intake once the animals achieved the 15th week. However, SHR maintained the same water intake over time (Table 1).

3.2. Optical redox ratio and UCP-1 from brown adipose tissue

To assess whether hypertension influences changes in adipose tissue metabolic profile, we examined the BAT from 6 to 15-week-old WIS, WKY and SHR animals (Fig. 1). At week 6, based on the raw FAD and NADH fluorescence lifetime images, the BAT of WIS rats exhibited high metabolic activity, i.e. more oxidized state (indicated in yellow-orange; Fig. 1A), similar to BAT from SHR. Redox ratio quantifications are described in Fig. 1C. In contrast, BAT of WKY animals presented lower metabolic activity (indicated by the green hues) with significant higher optical redox ratio compared to fat pad from the same location of WIS and SHR (Fig. 1C). Furthermore, BAT from WKY showed the largest lipid droplets when compared to SHR and WIS. Despite the contrast in metabolic activity, the strains did not exhibit any differences in UCP-1 protein expression (Fig. 1D-E/Supplementary Fig. 4). Panel B from Fig. 1 illustrates the differences in BAT after the development of hypertension in SHR, at week 15. At this age, the BAT from all strains became very similar to each other. BAT depots from WIS and SHR animals exhibited a decreased fluorescent decay rate with an increase in lipid droplets size and optical redox ratio (Fig. 1C). BAT from WKY, even with smaller yellowish lipid droplets, did not present differences in the optical redox ratio profile when compared to 6-week-old WKY animals (Fig. 1C). All strains presented increasing in UCP-1 protein levels over the time with no significant differences among the analyzed strains (Fig. 1D-E/Supplementary Fig. 4).

3.3. Optical redox ratio and UCP-1 from white adipose tissue

In order to evaluate the effect of hypertension in white adipose tissue (WAT), we examined the iWAT of 6 and 15-week-old WIS, WKY and SHR. Raw FAD and NADH fluorescence lifetime images showed

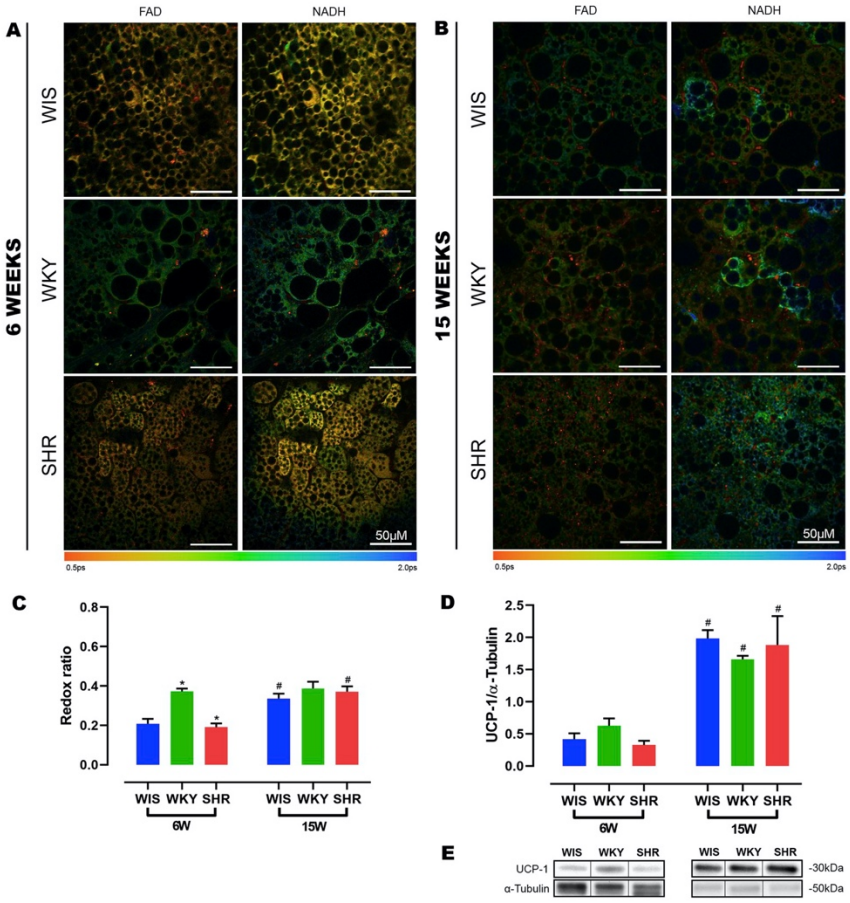

Fig. 1. Optical readouts of brown adipose tissue (BAT) for WIS, WKY and SHR. A) Representative maps of redox ratio at 6 weeks of age; B) Representative maps of redox ratio at 15 weeks of age; C) Means and SEMs of redox ratio at 6th and 15th week of age; D) UCP-1 expression at 6th and 15th week; E) Representative Western blots of UCP-1 and α-tubulin at 6th and 15th week. *$p < 0.05$ compared to control in the same age (WIS vs. WKY; WKY vs. SHR); $^{\#}p < 0.05$ compared between strains over the time. Full-length blots/gels are presented in Supplementary Fig. 4. WIS = Wistar; WKY = Wistar-Kyoto; SHR = Spontaneously Hypertensive Rats.

that all the strains present peripherally metabolic activity, but only WKY and SHR (Fig. 2A highlighted area) presented regions with increased fluorescence intensity and small lipid droplets, very similar to BAT phenotype (Fig. 2A), with no detectable UCP-1 protein expression (Fig. 2D/Supplementary Fig. 5). Despite the similarities, the iWAT from WKY rats exhibited a lower optical redox ratio than SHR and WIS strains (Fig. 2C). At week 15, iWAT from WIS and WKY rats exhibited a decreased fluorescent decay rate, leading to an increase in optical redox ratio when compared to the younger counterparts (Fig. 2B–C). In addition, iWAT from WKY did not show any regions with increased metabolic activity with optical redox ratio very similar to WIS (Fig. 2C). iWAT from SHR maintained the same metabolic profile over time, exhibiting several regions with brown-like adipocytes (Fig. 2B–C). No detectable UCP-1 protein expression was observed at this age (Fig. 2D/Supplementary Fig. 5).

3.4. Hormones and glycaemia measurement

We next sought to examine possible hormones connected intrinsically to metabolism. Serum TSH levels were significantly elevated in SHR when compared to WKY at week 6, but there were no differences over time. In addition, WIS and WKY reached similar values at week 6 and 15. Serum TSH concentration was not significantly different at any age between the strains. Serum T3 was not significantly different between strains at 6 weeks of age. T3 levels from WIS and WKY strains did not show any significant alteration at 15 weeks of age. Interestingly, SHR animals exhibited an increase on T3 serum level when compared to WKY rats of the same age.

WKY animals exhibited higher ACTH levels when compared to the other strains in both ages. However, at 15 weeks of age, a significant decrease of ACTH levels was observed for all strains. Corticosterone did not show any significant alteration over the time or between strains (Table 2). To confirm that the animals were not under metabolic stress, glycemic levels were measured at the beginning of the experiments and all strains presented values lower than 100 mg/dL (fasted awakened rats). SHR animals presented lower values than those observed for WKY (WIS: 93.17 ± 5.37; WKY: 96 ± 3.89; SHR: 79.17 ± 3.53*).

3.5. Adipokines measurement

We also examined serum adipokines at 15-week-old animals. Leptin levels were lower in SHR when compared to the WKY strain, which was similar to WIS. WKY exhibited lower adiponectin levels when compared to WIS and SHR. Changes in blood pressure did not interfere in the production of these adipokines (Table 3).

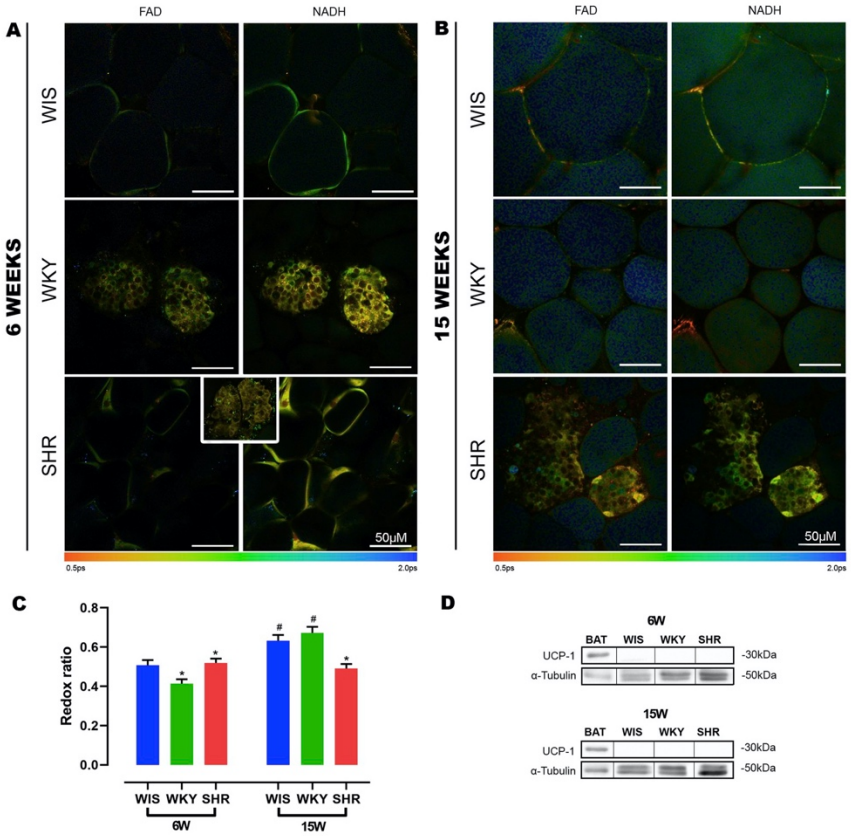

Fig. 2. Optical readouts of inguinal white adipose tissue (iWAT) for WIS, WKY and SHR. A) Representative maps of redox ratio at 6 weeks of age; B) Representative maps of redox ratio at 15 weeks of age; C) Means and SEMs of redox ratio at 6th and 15th week of age; D) Representative Western blots of UCP-1 and α-tubulin at 6th and 15th week. *p < 0.05 compared to control in the same age (WIS vs. WKY; WKY vs. SHR); #p < 0.05 compared between strains over the time. Full-length blots/gels are presented in Supplementary Fig. 5. WIS = Wistar; WKY = Wistar-Kyoto; SHR = Spontaneously Hypertensive Rats.

4. Discussion

An important characteristic of the AT is its enormous plasticity for volume, cell-number and phenotype variations (Penicaud et al., 2000). WAT represents the main energy reservoir of the body, while BAT is characterized by energy dissipation through thermogenesis and by the presence of the UCP-1 protein (Bargut et al., 2017; Marlatt and Ravussin, 2017). The UCP-1 is uniquely expressed in BAT, which uncouples the respiratory chain of oxidative phosphorylation within the mitochondria, shifts energy from the mitochondrial electron chain away from ATP production, and releases energy as heat (Montanari and Colitti, 2017; He et al., 2018).

Table 3
Serum values for leptin and adiponectin in WIS, WKY and SHR at 15 weeks of age.

	WIS	WKY	SHR
Leptin (pg/mL)	5452 ± 473.5	4820 ± 823.2	972.3 ± 260.2$
Adiponectin (ng/mL)	28534 ± 2619	14391 ± 729.8$	29897 ± 3256$

Table 3 – Serum values for leptin and adiponectin for WIS, WKY and SHR. Data are presented as mean ± SEM. $p < 0.05 compared to control at 15th week (WIS vs. WKY; WKY vs. SHR); WIS = Wistar; WKY = Wistar-Kyoto; SHR = Spontaneously Hypertensive Rats.

Table 2
Comparison of serum values for TSH, T3, ACTH and corticosterone in WIS, WKY and SHR animals at 6 and 15 weeks of age.

	6th WEEK			15th WEEK		
	WIS	WKY	SHR	WIS	WKY	SHR
TSH (ng/mL)	3.96 ± 1.35	4.88 ± 0.72	12.82 ± 2.93#	4.90 ± 0.66	6.38 ± 2.48	10.1 ± 3.89
T3 (pmol/L)	4570 ± 546.10	4272 ± 333.40	4041 ± 209.20	9086 ± 1321.00	8257 ± 1455.00	10265 ± 1701.00*
ACTH (pmol/L)	0.70 ± 0.09	1.56 ± 0.28#	0.86 ± 0.12#	0.41 ± 0.04*	0.61 ± 0.04*$	0.37 ± 0.04*$
Corticosterone (nmol/L)	458.80 ± 64.16	332.80 ± 35.62	395.90 ± 22.41	616.80 ± 93.51	450.00 ± 97.09	407.50 ± 47.47

Table 2 – Serum values for TSH, T3, ACTH and corticosterone for WIS, WKY and SHR. Data are presented as mean ± SEM. *p < 0.05 compared between strains over the time; #p < 0.05 compared to control at 6th week (WIS vs. WKY; WKY vs. SHR); $p < 0.05 compared to control at 15th week (WIS vs. WKY; WKY vs. SHR); WIS = Wistar; WKY = Wistar-Kyoto; SHR = Spontaneously Hypertensive Rats; TSH = Thyroid-stimulating hormone; T3 = Triiodothyronine; ACTH = Adrenocorticotropic hormone.

A direct evaluation of mitochondrial metabolic profile is the quantification of NADH and FAD production, which are highly involved in the mitochondrial fatty acid β-oxidation, the catabolic process of fatty acid molecules, and is crucial to energy dissipation of brown adipocytes (He et al., 2018). Thereby, redox ratio measure by FLIM becomes a powerful label-free approach to validate the metabolic activity of adipose tissue. We observed here that the different adipose pads exhibited distinct optical signatures, which reflected the differential energy consumption pattern associated with their function - BAT from these three different strains was characterized by a lower optical redox ratio and shorter fluorescence lifetime when compared to WAT from the same strains.

The involvement of the HPA axis that would trigger an environmental distress leading to the observed phenomenon was discarded, since the fasting plasma glucose for the three strains evaluated are consistent with fasting status (Mouri and Bhimji, 2017). Although, there are differences concerning glucose responsiveness in WKY and SHR, they are glucose intolerant, besides showing age-dependent increase in expression of the GLUT-4 in gastrocnemius muscle and heart, when compared to WIS rats (Alves-Wagner et al., 2014; Katayama et al., 1997). In this context, these strains exhibit impairment in insulin-induced glucose uptake, hyperinsulinemia, and a reduced rate of insulin removal from serum (Katayama et al., 1997; Mondon and Reaven, 1988). These studies might explain SHR lower glucose levels observed before anesthetic administration in awakened condition. However, in our experimental conditions, WKY did not present fasting hyperglycemic levels when compared with WIS rats, as previously described by Katayama et al. (1997). Additionally, the overstimulated HPA axis leads to the renin-angiotensin-aldosterone system (RAAS) activation but these events have not been described as the main cause of hypertension in SHR. Instead, previous studies have shown a clear correlation between the HPT axis and the development of hypertension in these animals, metabolic alterations and hypolipodystrophy (Schleiffer et al., 1981; Berta et al., 2019). The activation of the RAAS is also an important mediator of elevated blood pressure (Borghi et al., 2016), not only as an effect of sympathetic nervous system overactivity and renal compression but also dysfunctional adipose tissue (Schütten et al., 2017). The difference in metabolic profiles in different fat pads may reflect differentiated local activation of RAAS, since adipose tissue express all RAAS mediators (Borghi et al., 2016), but further investigation is needed to confirm this relationship.

Corroborating our findings in the rat strains analyzed, it is already known that over time brown adipocytes also become characteristically more like white adipocytes, increasing their intracellular lipid content and overall size (Sanchez-Gurmaches et al., 2016; Roberts-Toler et al., 2015; Schosserer et al., 2018). This phenomenon may be related to the decreased ACTH values found in these animals as this hormone plays an important role in regulating BAT activity and browning of WAT (van den Beukel et al., 2014; Schnabl et al., 2018; Ramage et al., 2016). Among the studied strains, WKY exhibited higher ACTH levels over time, which results in a BAT with a lower metabolic activity due to the fact that upraised ACTH levels acutely decrease BAT activity in rats (Ramage et al., 2016). A 4-fold increase in BAT metabolic activity was previously reported in lean individuals when compared to overweight/obese individuals (Marlatt and Ravussin, 2017; van Marken Lichtenbelt et al., 2009), indicating that the low body weight observed in SHR may be a result of milder whitening of BAT.

Adiponectin expression is poorly correlated with adiposity, showing low levels in obesity and insulin resistance (Meyer et al., 2013; Ahima et al., 2006) as circulating levels of leptin are highly correlated to body fat mass (Stern et al., 2016). Light weighted SHR presented lower leptin levels, and higher food intake. The inverted relationship of high adiposity and low levels of adiponectin confirms the literature data in normotensive animals. The lower values of leptin in SHR support the increased food intake of this strain and confirms the hypolipodystrophy phenotype showed by SHR.

The conversion of white pre-adipocytes into brown-like fat cells also called “brite” adipocytes or Brite/Beige adipose tissue, increase the presence of functional UCP1-rich cells in AT (Montanari and Colitti, 2017; Chen et al., 2016). Brite adipocytes were reported as a type of a WAT adipocytes but resembling brown adipocytes phenotype. In the basal state, brite adipocytes act as white adipocytes, but under the adequate stimulus, they might turn into brown-like adipocytes, characterizing the browning phenomenon (Bargut et al., 2017; Wu et al., 2012). The browning is mainly driven by chronic cold exposure, which triggers a sympathetic and TRH axis stimulation culminating with UCP-1 and other thermogenic proteins overexpression (Montanari and Colitti, 2017). The subcutaneous depots of WAT are the most common location for browning as these adipocytes are predominantly smaller and have a greater potential to differentiate (Bargut et al., 2017; Gustafson and Smith, 2015). The ectopic expression of UCP-1 is used to identify the presence of brite adipocytes within the white adipocytes (Bargut et al., 2017; Wu et al., 2013). We observed UCP-1 positive regions in the iWAT from 6-week-old SHR and WKY rats.

Over time, specific phenotypic patterns were observed for each animal strain: at week 15, the browning regions observed in iWAT from WKY animals underwent white adipocytes infiltration, distributing the brite adipocytes more diffusely, characterizing the whitening phenomenon. This change in distribution can be attributed to aging, which is directly associated with whole-body adipose tissue redistribution (Schosserer et al., 2018). Unlike WKY animals, iWAT from 15-week-old SHR preserved the browning regions, without any WAT permeation due to aging. This browning profile may be associated with the increased release and metabolism of norepinephrine characteristic of SHR adipose pad (Cabassi et al., 1996, 2002) and be associated with the higher T3 levels seen at week 15. The TRH axis may also play a crucial role in the SHR AT configuration observed. TSH enhances the differentiation of preadipocytes into adipocytes and activates crucial pathways in adipogenesis and preadipocyte survival (Comas et al., 2018). Thus, the increased TSH in SHR is linked to the increase sympathoadrenal activity (Judy et al., 1978; Kvetnansky et al., 1979; Garcia et al., 1995; García et al., 2002; Duntas and Brenta, 2012). This hormonal ambiance generates a tissue with a greater number of little lipid droplets when compared to other strains, by the recruitment of pre-adipocytes and changes in BAT redox ratio.

Additionally, it has been shown that certain WAT pads are less prone to browning (Walden et al., 2012; Lo and Sun, 2013). We observed that SHR iWAT is more susceptible to browning than the other pads when exposed to the same hormonal conditions (Fig. 2/Supplementary Figs. 1–3) (Lo and Sun, 2013; Seale et al., 2011).

In summary, we correlate adipose tissue whitening with changes in the metabolic profile of different types of adipose tissue pads over time. Further investigation is needed to confirm the possible effect of the TRH-T3 axis in the attenuation of metabolic changes. Also, the impact of the hormonal environment from hypertensive rats like SHR in maintenance of hypolipodystrophic and the correlation between absence of protective adipose molecules present in hypolipodystrophy condition and establishment of hypertension still need to be investigated. Describing the hormonal imbalance caused by hypertension along with the relationship between hypolipodystrophy and hypertension can highlight key molecules in the development of novel and innovative therapeutic approaches to adiposity dysfunctions and hypertension treatment.

CRediT authorship contribution statement

Filipy Borghi: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Supervision, Validation, Visualization, Writing - original draft, Writing - review & editing. **Carolina Silva:** Formal analysis, Investigation, Validation, Visualization, Writing - original draft. **Priscila Cristina da Silva:** Investigation, Project administration, Supervision, Writing - original

draft. **Danilo Lopes Ferrucci:** Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization. **Camila Lidiane Morais:** Data curation, Formal analysis, Investigation, Visualization. **Ana Gabriela Conceição-Vertamatti:** Formal analysis, Investigation, Methodology, Validation. **Hernandes Faustino Carvalho:** Methodology, Supervision. **Matheus de Castro Fonseca:** Data curation, Formal analysis, Investigation, Methodology, Writing - original draft. **André Schwambach Vieira:** Methodology, Supervision, Visualization. **Dora Maria Grassi-Kassisse:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Validation, Visualization, Writing - original draft, Writing - review & editing.

Declaration of competing interest

The authors declare no competing interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mce.2020.110758.

References

Adur, J., Pelegati, V.B., de Thomaz, A.A., et al., 2012. Quantitative changes in human epithelial cancers and osteogenesis imperfecta disease detected using nonlinear multicontrast microscopy. J. Biomed. Optic. 17 (8) 081407-081401.

Ahima, R.S., Qi, Y., Singhal, N.S., Jackson, M.B., Scherer, P.E., 2006. Brain adipocytokine action and metabolic regulation. Diabetes 55 (Suppl. 2), S145–S154.

Akinci, B., Meral, R., Oral, E.A., 2018. Phenotypic and genetic characteristics of lipodystrophy: pathophysiology, metabolic abnormalities, and comorbidities. Curr. Diabetes Rep. 18 (12).

Alonzo, C.A., Karaliota, S., Pouli, D., Liu, Z., Karalis, K.P., Georgakoudi, I., 2016. Two-photon excited fluorescence of intrinsic fluorophores enables label-free assessment of adipose tissue function. Sci. Rep. 6, 31012.

Alves-Wagner, A.B., Sabino-Silva, R., Campello, R.S., Mori, R.C., Machado, U.F., 2014. Decreased diabetes-induced glycemic impairment in WKY and SHR involves enhanced skeletal muscle Slc2a4/GLUT4 expression. Diabetol. Metab. Syndrome 6 (1), 97.

Arbeeny, C.M., Meyers, D.S., Hillyer, D.E., Bergquist, K.E., 1995. Metabolic alterations associated with the antidiabetic effect of beta 3-adrenergic receptor agonists in obese mice. Am. J. Physiol. 268 (4 Pt 1), E678–E684.

Bargut, T.C., Souza-Mello, V., Aguila, M.B., Mandarim-de-Lacerda, C.A., 2017. Browning of white adipose tissue: lessons from experimental models. Horm. Mol. Biol. Clin. Invest.

Bartelt, A., Bruns, O.T., Reimer, R., et al., 2011. Brown adipose tissue activity controls triglyceride clearance. Nat. Med. 17 (2), 200–205.

Berg, T., 2014. β3-adrenoceptors inhibit stimulated norepinephrine release in spontaneously hypertensive rats. Front. Physiol. 5, 499.

Berg, T., 2015. Altered β1-3-adrenoceptor influence on α2-adrenoceptor-mediated control of catecholamine release and vascular tension in hypertensive rats. Front. Physiol. 6, 120.

Berta, E., Lengyel, I., Halmi, S., et al., 2019. Hypertension in thyroid disorders. Front. Endocrinol. 10.

Bianco, A.C., Sheng, X.Y., Silva, J.E., 1988. Triiodothyronine amplifies norepinephrine stimulation of uncoupling protein gene transcription by a mechanism not requiring protein synthesis. J. Biol. Chem. 263 (34), 18168–18175.

Borghi, F., Sevá-Pessôa, B., Grassi-Kassisse, D.M., 2016. The adipose tissue and the involvement of the renin-angiotensin-aldosterone system in cardiometabolic syndrome. Cell Tissue Res. 366 (3), 543–548.

Borghi, F., Morais, C.L., Silva, C., et al., 2019. A new perspective of lactatogenesis by isolated adipocytes. Mol. Cell. Endocrinol. 498, 110560.

Marlatt, Kara L., Ravussin, Eric, 2017. Brown Adipose tissue: an update on recent findings. - PubMed - NCBI. Curr. Obes. Rep.

Cabassi, A., Bergamaschi, E., Mutti, A., Franchini, I., Borghetti, A., 1996. Age-related changes in interstitial norepinephrine. A microdialysis study in spontaneously hypertensive rats. Am. J. Hypertens. 9 (9), 878–883.

Cabassi, A., Vinci, S., Cantoni, A.M., et al., 2002. Sympathetic activation in adipose tissue and skeletal muscle of hypertensive rats. Hypertension 39 (2 Pt 2), 656–661.

Campbell, D.J., Duncan, A.M., Kladis, A., Harrap, S.B., 1995. Angiotensin peptides in spontaneously hypertensive and normotensive Donryu rats. Hypertension 25 (5), 928–934.

Cannon, B., Nedergaard, J., 2004. Brown adipose tissue: function and physiological significance. Physiol. Rev. 84 (1), 277–359.

Chance, B., Schoener, B., Oshino, R., Itshak, F., Nakase, Y., 1979. Oxidation-reduction ratio studies of mitochondria in freeze-trapped samples. NADH and flavoprotein fluorescence signals. J. Biol. Chem. 254 (11), 4764–4771.

Chen, Y., Pan, R., Pfeifer, A., 2016. Fat tissues, the brite and the dark sides. Pflügers Archiv.

Comas, F., Lluch, A., Sabater, M., et al., 2018. Adipose tissue TSH as a new modulator of human adipocyte mitochondrial function. Int. J. Obes. 1.

Conceicao-Vertamatti, A.G., Borghi, F., Canova, F., Grassi-Kassisse, D.M., 2017. History of vascular reactivity models and their involvement in hypertension pathogenesis. Vasa 1–9.

Das, E., Moon, J.H., Lee, J.H., Thakkar, N., Pausova, Z., Sung, H.K., 2018. Adipose tissue and modulation of hypertension. Curr. Hypertens. Rep. 20 (11), 96.

Digman, M.A., Caiolfa, V.R., Zamai, M., Gratton, E., 2008. The phasor approach to fluorescence lifetime imaging analysis. Biophys. J. 94 (2), L14–L16.

Duntas, L.H., Brenta, G., 2012. The effect of thyroid disorders on lipid levels and metabolism. Med. Clin. 96 (2), 269–281.

Fite, B.Z., Decaris, M., Sun, Y., et al., 2011. Noninvasive multimodal evaluation of bioengineered cartilage constructs combining time-resolved fluorescence and ultrasound imaging. *Tissue engineering Part C.* Methods 17 (4).

Garcia, S.I., Dabsys, S.M., Martinez, V.N., et al., 1995. Thyrotropin-releasing hormone hyperactivity in the preoptic area of spontaneously hypertensive rats. Hypertension 26 (6 Pt 2), 1105–1110.

García, S.I., Landa, M.S., Porto, P.I., et al., 2002. Thyrotropin-releasing hormone decreases leptin and mediates the leptin-induced pressor effect. Hypertension 39 (2 Pt 2), 491–495.

Gulledge, C.J., Dewhirst, M.W., 1996. Tumor oxygenation: a matter of supply and demand. Anticancer Res. 16 (2), 741–749.

Gustafson, B., Smith, U., 2015. Regulation of white adipogenesis and its relation to ectopic fat accumulation and cardiovascular risk. Atherosclerosis 241 (1), 27–35.

He, S., An, Y., Li, X., et al., 2018. In vivo metabolic imaging and monitoring of brown and beige fat. J. Biophot.

Hernandez, A., de Mena, R.M., Martin, E., Obregon, M.J., 2011. Differences in the response of UCP1 mRNA to hormonal stimulation between rat and mouse primary cultures of brown adipocytes. Cell. Physiol. Biochem. 28 (5), 969–980.

Hill, B.G., 2015. Insights into an adipocyte whitening program. Adipocyte 4 (1), 75–80.

Huang, S., Heikal, A.A., Webb, W.W., 2002. Two-photon fluorescence spectroscopy and microscopy of NAD(P)H and flavoprotein. Biophys. J. 82 (5), 2811–2825.

Jones, J.D., Ramser, H.E., Woessner, A.E., Quinn, K.P., 2018. In vivo multiphoton microscopy detects longitudinal metabolic changes associated with delayed skin wound healing. Commun. Biol. 1 (1), 1–8.

Judy, W.V., Watanabe, A.M., Henry, D.P., Besch, H.R., Aprison, B., 1978. Effect of l-dopa on sympathetic nerve activity and blood pressure in the spontaneously hypertensive rat. Circ. Res. 43 (1), 24–28.

Katayama, S., Inaba, M., Maruno, Y., Morita, T., Awata, T., Oka, Y., 1997. Glucose intolerance in spontaneously hypertensive and Wistar-Kyoto rats: enhanced gene expression and synthesis of skeletal muscle glucose transporter 4. Hypertens. Res. 20 (4), 279–286.

Kvetnansky, R., McCarty, R., Thoa, N.B., Lake, C.R., Kopin, I.J., 1979. Sympatho-adrenal responses of spontaneously hypertensive rats to immobilization stress. Am. J. Physiol. 236 (3), H457–H462.

Lee G, Goosens KA. Sampling blood from the lateral tail vein of the Rat2015;No. 99. Located at: J. Vis. Exp..

Lee, M.J., Wu, Y., Fried, S.K., 2013. Adipose tissue heterogeneity: implication of depot differences in adipose tissue for obesity complications. Mol. Aspect. Med. 34 (1), 1–11.

Leong, X.F., Ng, C.Y., Jaarin, K., 2015. Animal models in cardiovascular research: hypertension and atherosclerosis. BioMed Res. Int. 2015, 528757.

Lo, K.A., Sun, L., 2013. Turning WAT into BAT: a review on regulators controlling the browning of white adipocytes. Biosci. Rep. 33 (5).

Ma, X., Lee, P., Chisholm, D.J., James, D.E., 2015. Control of adipocyte differentiation in different fat depots; implications for pathophysiology or therapy. Front. Endocrinol. 6, 1.

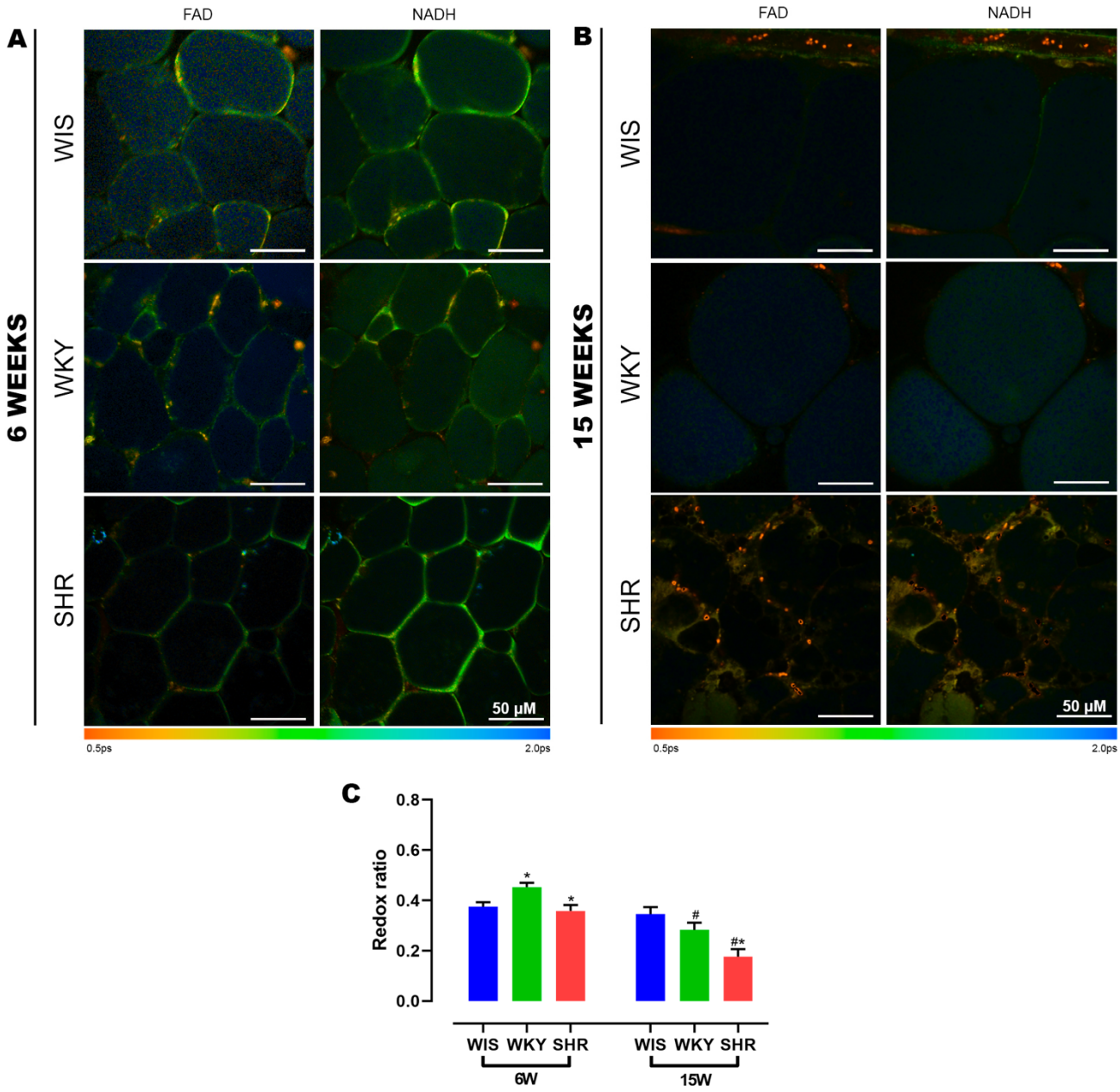
MacLean, P.S., Higgins, J.A., Giles, E.D., Sherk, V.D., Jackman, M.R., 2015. The role for adipose tissue in weight regain after weight loss. Obes. Rev. 16 (Suppl. 1), 45–54.

Mann, A., Thompson, A., Robbins, N., Blomkalns, A.L., 2014. Localization, identification, and excision of murine adipose depots. J. Vis. Exp. 94.

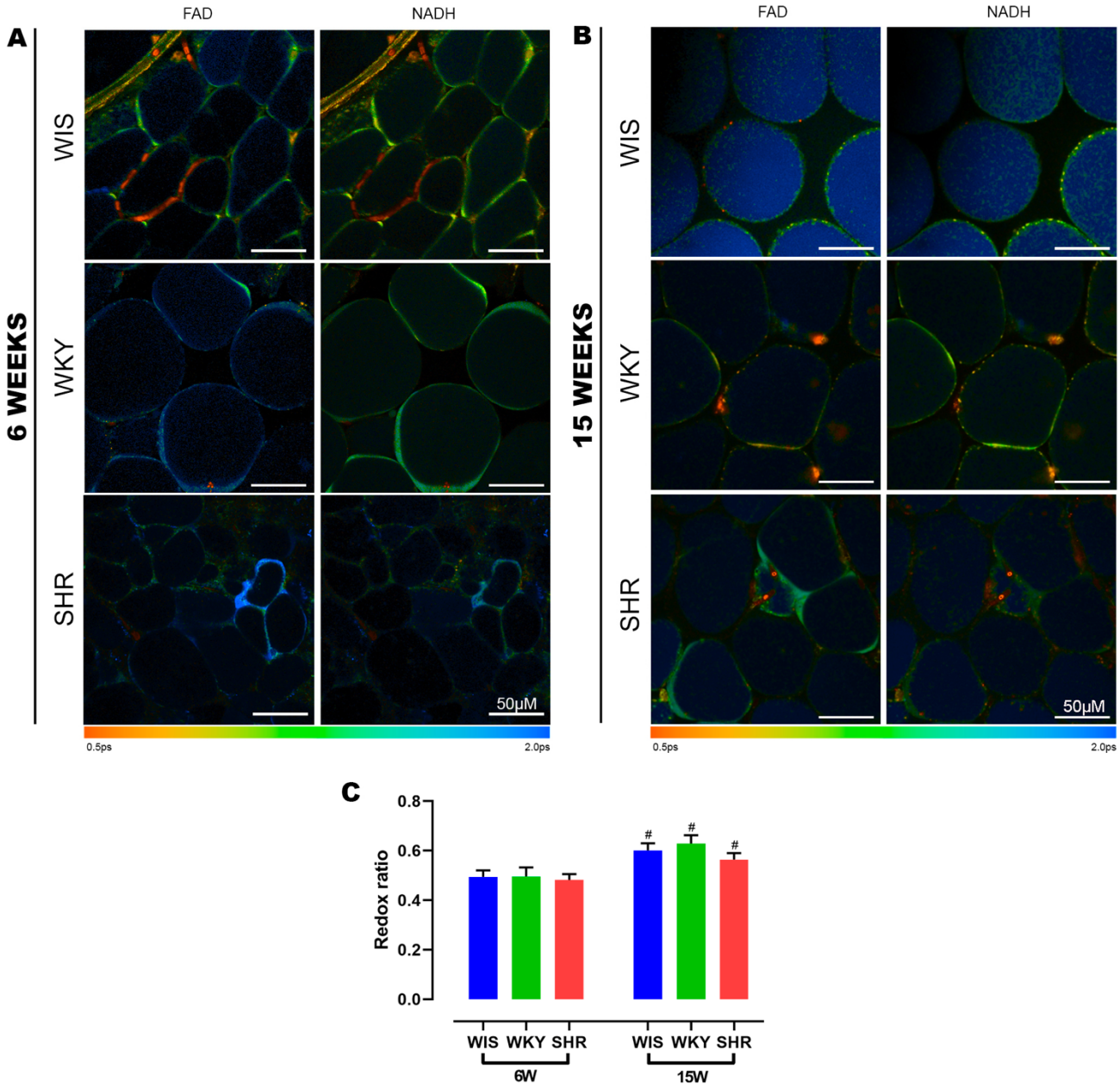
Marlatt, K.L., Ravussin, E., 2017. Brown adipose tissue: an update on recent findings. Curr. Obes. Rep. 6 (4), 389–396.

Meyer, L.K., Ciaraldi, T.P., Henry, R.R., Wittgrove, A.C., Phillips, S.A., 2013. Adipose tissue depot and cell size dependency of adiponectin synthesis and secretion in human obesity. Adipocyte 2 (4), 217–226.
Mondon, C.E., Reaven, G.M., 1988. Evidence of abnormalities of insulin metabolism in rats with spontaneous hypertension. Metabolism 37 (4), 303–305.
Montanari, T.P.N., Colitti, M., 2017. Factors involved in white-to-brown adipose tissue conversion and in thermogenesis: a review. - PubMed - NCBI. Obes. Rev. 18 (5), 495–513.
Morais, C.L., Borghi, F., Silva, C., da Silva, P.C., Grassi-Kassisse, D.M., 2017. Relation of morphometry and basal lactate production in primary culture of isolated adipocytes from normotensive and hypertensive rats. In: XXV Congresso de Iniciação Científica da Unicamp. PIBIC, Campinas.
Mouri, M., Bhimji, S., 2017. *Hyperglycemia.* StatPearls. StatPearls Publishing LLC.
Okamoto, K., Aoki, K., 1963. Development of a strain of spontaneously hypertensive rats. Jpn. Circ. J. 27, 282–293.
Palmer, A.K., Kirkland, J.L., 2016. Aging and adipose tissue: potential interventions for diabetes and regenerative medicine. Exp. Gerontol. 86, 97–105.
Peirce, V., Carobbio, S., Vidal-Puig, A., 2014. The different shades of fat. Nature 510 (7503), 76–83.
Pelegati, V.B., Adur, J., Thomaz, AAd, et al., 2012. Multimodal optical setup for nonlinear and fluorescence lifetime imaging microscopies: improvement on a commercial confocal inverted microscope. Proc. SPIE.
Pellegrinelli, V., Carobbio, S., Vidal-Puig, A., 2016. Adipose tissue plasticity: how fat depots respond differently to pathophysiological cues. Diabetologia 59 (6), 1075–1088.
Penicaud, L., Cousin, B., Leloup, C., Lorsignol, A., Casteilla, L., 2000. The autonomic nervous system, adipose tissue plasticity, and energy balance. Nutrition 16 (10), 903–908.
Ramage, L.E., Akyol, M., Fletcher, A.M., et al., 2016. Glucocorticoids acutely increase Brown adipose tissue activity in humans, revealing species-specific differences in UCP-1 regulation. Cell Metabol. 24 (1), 130–141.
Ramanujam, N., Richards-Kortum, R., Thomsen, S., Mahadevan-Jansen, A., Follen, M., Chance, B., 2001. Low temperature fluorescence imaging of freeze-trapped human cervical tissues. Optic Express 8 (6), 335–343.
Roberts-Toler, C., O'Neill, B.T., Cypess, A.M., 2015. Diet-induced obesity causes insulin resistance in mouse brown adipose tissue. Obesity 23 (9), 1765–1770.
Saito, M., Okamatsu-Ogura, Y., Matsushita, M., et al., 2009. High incidence of metabolically active brown adipose tissue in healthy adult humans: effects of cold exposure and adiposity. Diabetes 58 (7), 1526–1531.
Sanchez-Gurmaches, J., Guertin, D.A., 2014. Adipocyte lineages: tracing back the origins of fat. Biochim. Biophys. Acta 1842 (3), 340–351.
Sanchez-Gurmaches, J., Hung, C.M., Guertin, D.A., 2016. Emerging complexities in adipocyte origins and identity. Trends Cell Biol. 26 (5), 313–326.
Scherer, P.E., 2006. Adipose tissue: from lipid storage compartment to endocrine organ. Diabetes 55 (6), 1537–1545.
Schleiffer, R., Berthelot, A., Pernot, F., Gairard, A., 1981. Parathyroids, thyroid and development of hypertension in SHR. Jpn. Circ. J. 45 (11).
Schnabl, K., Westermeier, J., Li, Y., Klingenspor, M., 2018. Opposing actions of adrenocorticotropic hormone and glucocorticoids on UCP1-mediated respiration in Brown adipocytes. Front. Physiol. 9, 1931.
Schosserer, M., Grillari, J., Wolfrum, C., Scheideler, M., 2018. Age-induced changes in white, brite, and Brown adipose depots: a mini-review. Gerontology 64 (3), 229–236.
Schütten, M.T., Houben, A.J., de Leeuw, P.W., Stehouwer, C.D., 2017. The link between adipose tissue renin-angiotensin-aldosterone system signaling and obesity-associated hypertension. Physiology 32 (3).
Seale, P., Conroe, H.M., Estall, J., et al., 2011. Prdm16 determines the thermogenic program of subcutaneous white adipose tissue in mice. J. Clin. Invest. 121 (1), 96–105.
Skala, M., Ramanujam, N., 2010. Multiphoton redox ratio imaging for metabolic monitoring in vivo. Methods Mol. Biol. 594, 155–162.
Skala, M.C., Riching, K.M., Gendron-Fitzpatrick, A., et al., 2007. In vivo multiphoton microscopy of NADH and FAD redox states, fluorescence lifetimes, and cellular morphology in precancerous epithelia. Proc. Natl. Acad. Sci. U. S. A. 104 (49), 19494–19499.
Stern, J.H., Rutkowski, J.M., Scherer, P.E., 2016. Adiponectin, leptin, and fatty acids in the maintenance of metabolic homeostasis through adipose tissue crosstalk. Cell Metabol. 23 (5), 770–784.
van den Beukel, J.C., Grefhorst, A., Quarta, C., et al., 2014. Direct activating effects of adrenocorticotropic hormone (ACTH) on brown adipose tissue are attenuated by corticosterone. Faseb. J. 28 (11), 4857–4867.
van Marken Lichtenbelt, W.D., Vanhommerig, J.W., Smulders, N.M., et al., 2009. Cold-activated brown adipose tissue in healthy men. N. Engl. J. Med. 360 (15), 1500–1508.
Virtanen, K.A., Lidell, M.E., Orava, J., et al., 2009. Functional brown adipose tissue in healthy adults. N. Engl. J. Med. 360 (15), 1518–1525.
Walden, T.B., Hansen, I.R., Timmons, J.A., Cannon, B., Nedergaard, J., 2012. Recruited vs. nonrecruited molecular signatures of brown, "brite," and white adipose tissues. Am. J. Physiol. Endocrinol. Metab. 302 (1), E19–E31.
Wang, W., Seale, P., 2016. Control of brown and beige fat development. Nat. Rev. Mol. Cell Biol.
Wang, C.Z., Wei, D., Guan, M.P., Xue, Y.M., 2014. Triiodothyronine regulates distribution of thyroid hormone receptors by activating AMP-activated protein kinase in 3T3-L1 adipocytes and induces uncoupling protein-1 expression. Mol. Cell. Biochem. 393 (1–2), 247–254.
Wu, J., Bostrom, P., Sparks, L.M., et al., 2012. Beige adipocytes are a distinct type of thermogenic fat cell in mouse and human. Cell 150 (2), 366–376.
Wu, J., Cohen, P., Spiegelman, B.M., 2013. Adaptive thermogenesis in adipocytes: is beige the new brown? Genes Dev. 27 (3), 234–250.
Zheng, Z., Liu, X., Zhao, Q., Zhang, L., Li, C., Xue, Y., 2014. Regulation of UCP1 in the browning of epididymal adipose tissue by β3-adrenergic agonist: a role for MicroRNAs. Internet J. Endocrinol. 2014, 530636.
Zhou, J., Qin, G., 2012. Adipocyte dysfunction and hypertension. Am. J. Cardiovasc. Dis. 2 (2), 143–149.

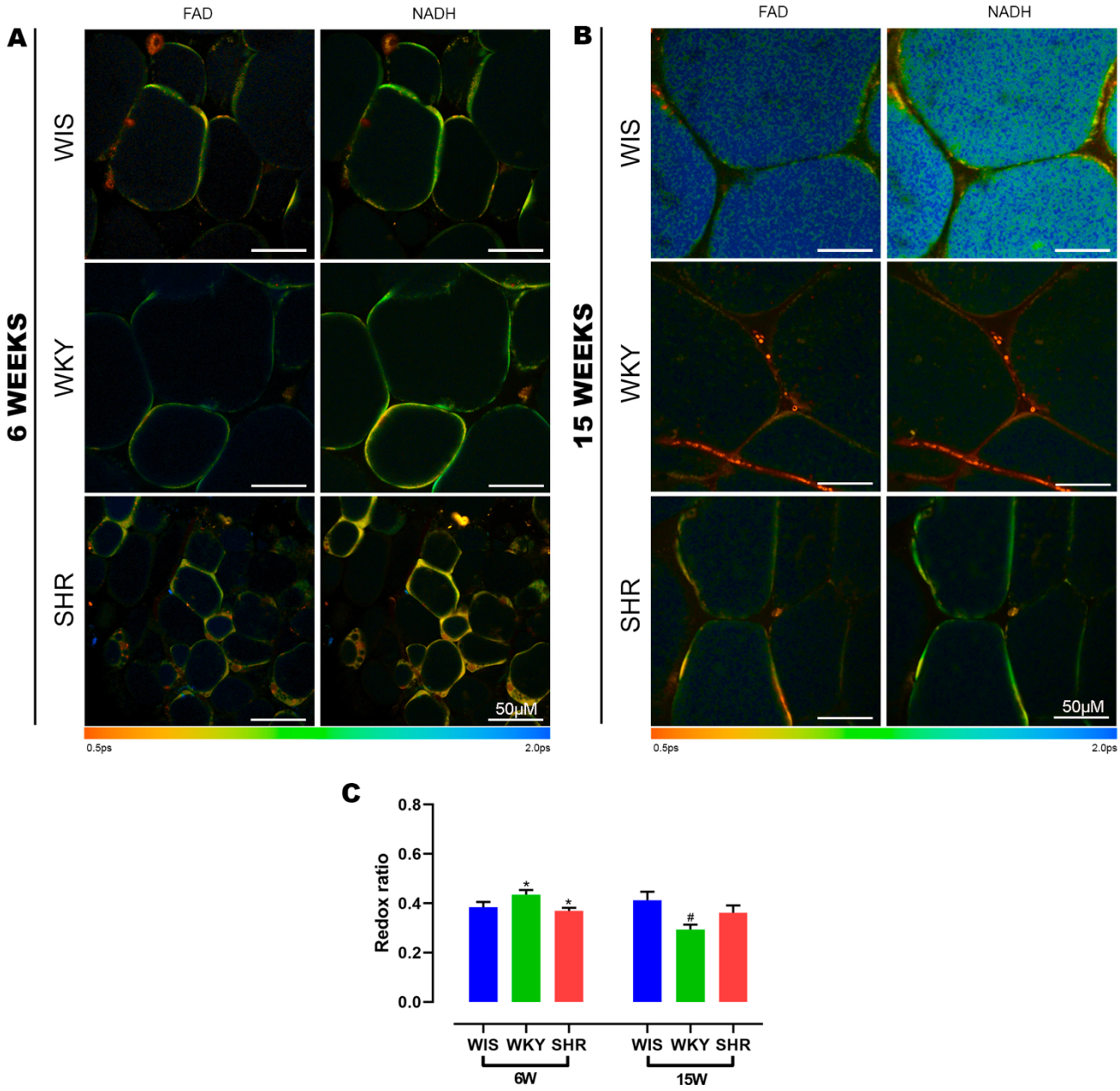
Appendix A. Supplementary data



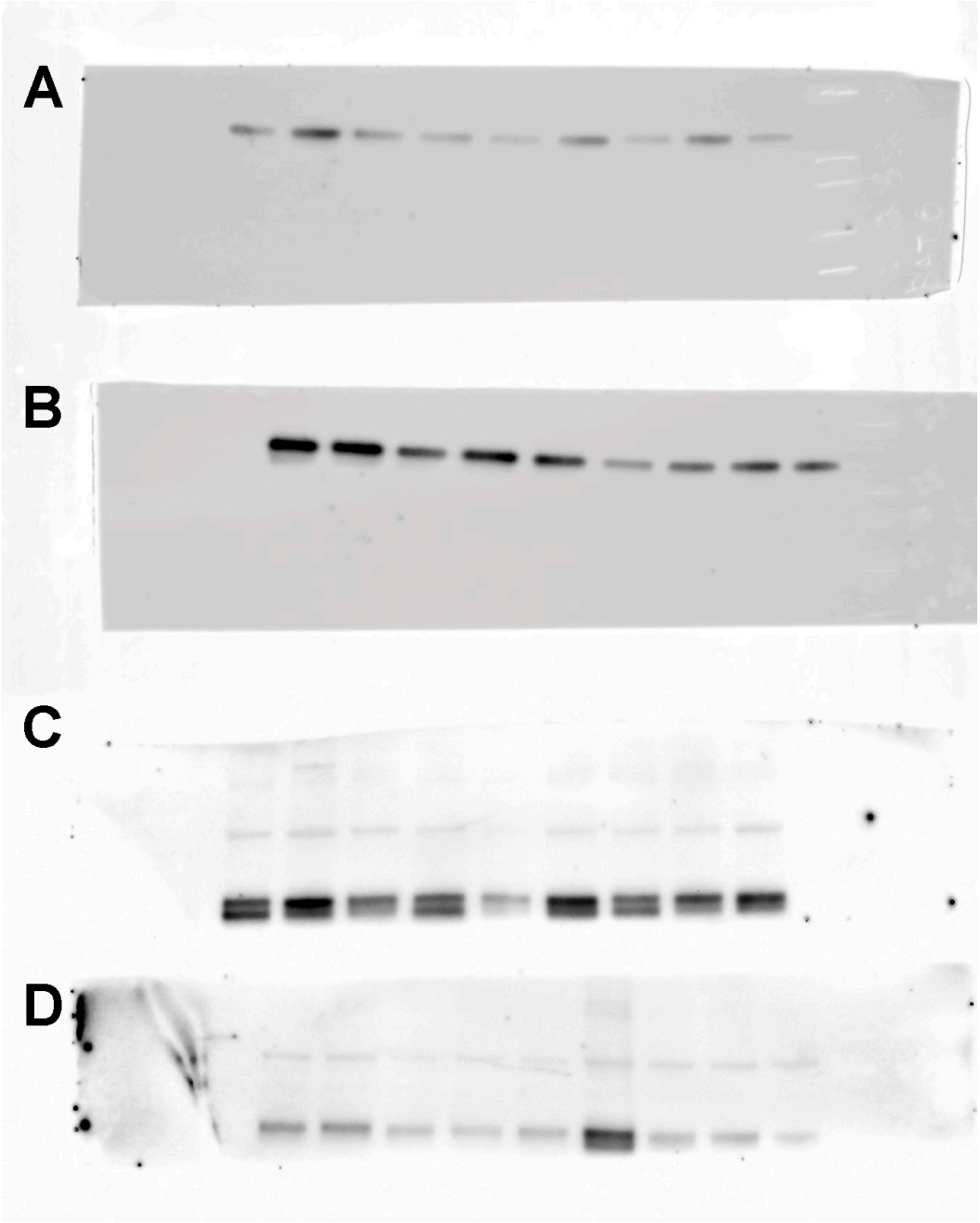
Supplementary Figure 1 – Optical readouts of epididymal white adipose tissue (eWAT) for WIS, WKY and SHR. A) Representative maps of redox ratio at 6 weeks of age; B) Representative maps of redox ratio at 15 weeks of age; C) Means and SEMs of redox ratio at 6th and 15th week of age. *p<0.05 compared to control in the same age (WIS vs. WKY; WKY vs. SHR); $^{\#}$p<0.05 compared between strains over the time. WIS = Wistar; WKY = Wistar-Kyoto; SHR = Spontaneously Hypertensive Rats.



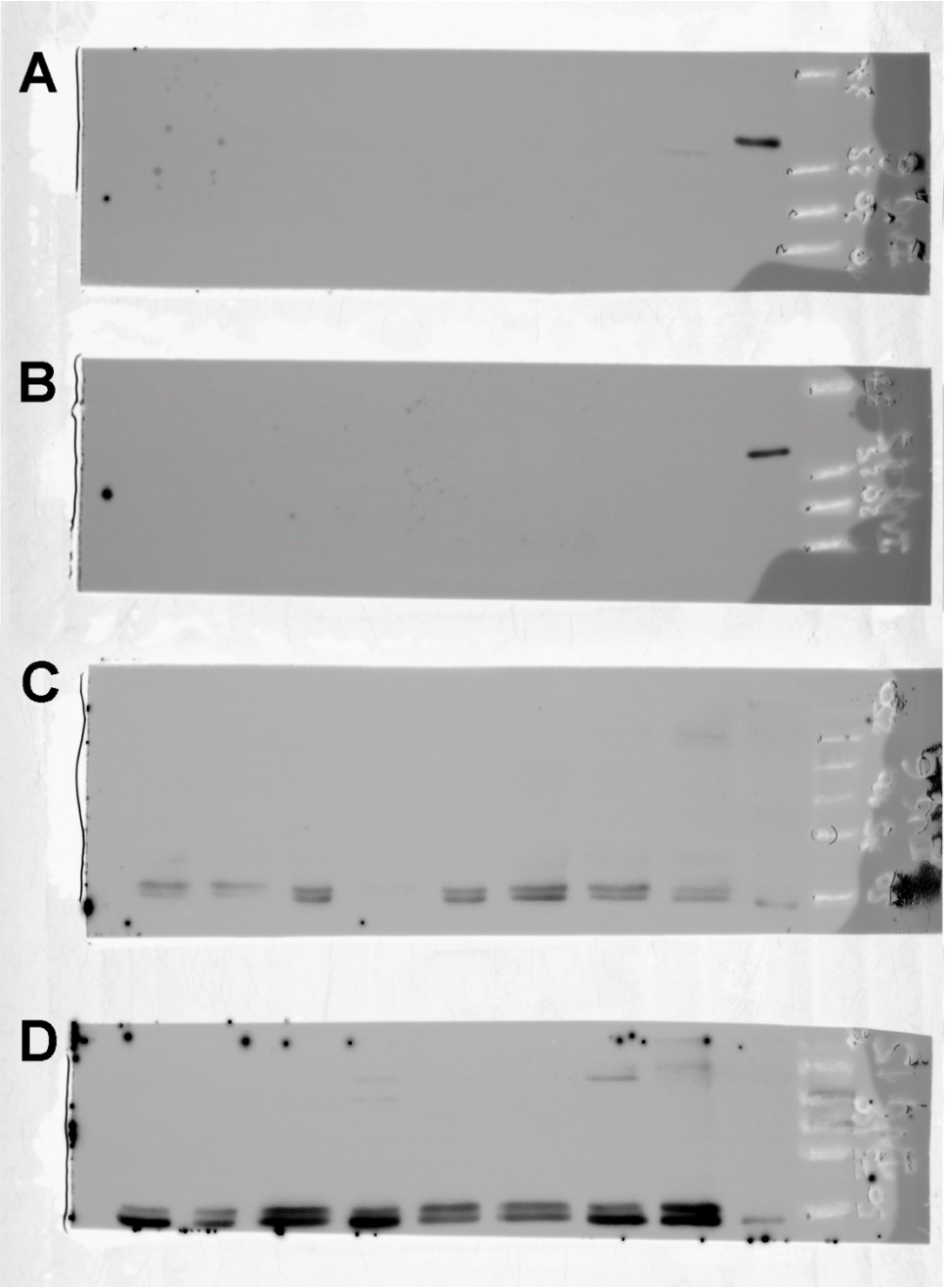
Supplementary Figure 2 – Optical readouts of mesenteric white adipose tissue (mWAT) for WIS, WKY and SHR. A) Representative maps of redox ratio at 6 weeks of age; B) Representative maps of redox ratio at 15 weeks of age; C) Means and SEMs of redox ratio at 6th and 15th week of age. $^{\#}p<0.05$ compared between strains over the time. WIS = Wistar; WKY = Wistar-Kyoto; SHR = Spontaneously Hypertensive Rats.



Supplementary Figure 3 – Optical readouts of retroperitoneal white adipose tissue (rWAT) for WIS, WKY and SHR. A) Representative maps of redox ratio at 6 weeks of age; B) Representative maps of redox ratio at 15 weeks of age; C) Means and SEMs of redox ratio at 6th and 15th week of age. *p<0.05 compared to control in the same age (WIS vs. WKY; WKY vs. SHR); $^{\#}$p<0.05 compared between strains over the time. WIS = Wistar; WKY = Wistar-Kyoto; SHR = Spontaneously Hypertensive Rats.



Supplementary Figure 4 – Full-length blots/gels from BAT for WIS, WKY and SHR. A) UCP-1 at 6 weeks of age; B) UCP-1 at 15 weeks of age; C) α-tubulin at 6 weeks of age; D) α-tubulin at 15 weeks of age. WIS = Wistar; WKY = Wistar-Kyoto; SHR = Spontaneously Hypertensive Rats.



Supplementary Figure 5 – Full-length blots/gels from iWAT for WIS, WKY and SHR. A) UCP-1 at 6 weeks of age; B) UCP-1 at 15 weeks of age; C) α-tubulin at 6 weeks of age; D) α-tubulin at 15 weeks of age. WIS = Wistar; WKY = Wistar-Kyoto; SHR = Spontaneously Hypertensive Rats.

CONCLUSÃO

O presente estudo descreve diferenças intrínsecas no perfil adiposo relacionadas à instalação da hipertensão ao longo do tempo. A tendência natural dos panículos adiposos é diminuir a sua atividade metabólica com o tempo, aumentando a sua relação redox. Com este trabalho, foi demonstrado que o ambiente hipertensivo vivenciado pelo SHR retardou este processo quando comparado aos controles normotensos, destacando o eixo TRH-T3 como possível modulador deste fenômeno e da hipolipodistrofia observada nestes animais. Além disso, em nossas condições experimentais não observamos influência relevante da AII na atividade lipolítica e/ou glicolítica de adipócitos isolados.

REFERÊNCIAS

Adams, C., A. Stears, D. Savage & C. Deaton (2018) "We're stuck with what we've got": The impact of lipodystrophy on body image. *J Clin Nurs*.

Ahmed, K., S. Tunaru, C. Tang, M. Müller, A. Gille, A. Sassmann, J. Hanson & S. Offermanns (2010) An autocrine lactate loop mediates insulin-dependent inhibition of lipolysis through GPR81. *Cell Metab,* 11**,** 311-9.

Arbeeny, C. M., D. S. Meyers, D. E. Hillyer & K. E. Bergquist (1995) Metabolic alterations associated with the antidiabetic effect of beta 3-adrenergic receptor agonists in obese mice. *Am J Physiol,* 268**,** E678-84.

Atlas, S. A. (2007) The renin-angiotensin aldosterone system: pathophysiological role and pharmacologic inhibition. *J Manag Care Pharm,* 13**,** 9-20.

Axelrod, J. & T. D. Reisine (1984) Stress hormones: their interaction and regulation. *Science,* 224**,** 452-9.

Bartelt, A., O. T. Bruns, R. Reimer, H. Hohenberg, H. Ittrich, K. Peldschus, M. G. Kaul, U. I. Tromsdorf, H. Weller, C. Waurisch, A. Eychmüller, P. L. Gordts, F. Rinninger, K. Bruegelmann, B. Freund, P. Nielsen, M. Merkel & J. Heeren (2011) Brown adipose tissue activity controls triglyceride clearance. *Nat Med,* 17**,** 200-5.

Baumann, M., R. Megens, R. Bartholome, S. Dolff, M. A. van Zandvoort, J. F. Smits, H. A. Struijker-Boudier & J. G. De Mey (2007) Prehypertensive renin-angiotensin-aldosterone system blockade in spontaneously hypertensive rats ameliorates the loss of long-term vascular function. *Hypertens Res,* 30**,** 853-61.

Berecek, K. H., S. Nagahama & S. Oparil (1984) Effect of central administration of MK-422 (the diacid form of enalapril) on the development of hypertension in the spontaneously hypertensive rat. *J Hypertens Suppl,* 2**,** S63-6.

Berg, T. (2014) β3-adrenoceptors inhibit stimulated norepinephrine release in spontaneously hypertensive rats. *Front Physiol,* 5**,** 499.

--- (2015) Altered β1-3-adrenoceptor influence on α2-adrenoceptor-mediated control of catecholamine release and vascular tension in hypertensive rats. *Front Physiol,* 6**,** 120.

--- (2016) α2-Adrenoreceptor Constraint of Catecholamine Release and Blood Pressure Is Enhanced in Female Spontaneously Hypertensive Rats. - PubMed - NCBI. *Frontiers in Neuroscience,* 10.

Bernatova, I. (2014) Endothelial dysfunction in experimental models of arterial hypertension: cause or consequence? *Biomed Res Int,* 2014**,** 598271.

Borghi, F., B. Sevá-Pessôa & D. M. Grassi-Kassisse (2016) The adipose tissue and the involvement of the renin-angiotensin-aldosterone system in cardiometabolic syndrome. *Cell Tissue Res*.

Boschmann, M., F. Adams, K. Schaller, G. Franke, A. M. Sharma, S. Klaus, F. C. Luft & J. Jordan (2006a) Hemodynamic and metabolic responses to interstitial angiotensin II in normal weight and obese men. *J Hypertens,* 24**,** 1165-71.

Boschmann, M., S. Engeli, F. Adams, G. Franke, F. C. Luft, A. M. Sharma & J. Jordan (2006b) Influences of AT1 receptor blockade on tissue metabolism in obese men. *Am J Physiol Regul Integr Comp Physiol,* 290**,** R219-23.

Boschmann, M., J. Jordan, F. Adams, N. J. Christensen, J. Tank, G. Franke, M. Stoffels, A. M. Sharma, F. C. Luft & S. Klaus (2003) Tissue-specific response to interstitial angiotensin II in humans. *Hypertension,* 41**,** 37-41.

Boschmann, M., J. Jordan, S. Schmidt, F. Adams, F. C. Luft & S. Klaus (2002) Gender-specific response to interstitial angiotensin II in human white adipose tissue. *Horm Metab Res,* 34**,** 726-30.

Boschmann, M., J. Ringel, S. Klaus & A. M. Sharma (2001) Metabolic and hemodynamic response of adipose tissue to angiotensin II. *Obes Res,* 9**,** 486-91.

Briasoulis, A. & G. L. Bakris (2013) The OSCAR for cardiovascular disease prevention in chronic kidney disease goes to blood pressure control. *Kidney Int,* 83**,** 20-2.

Briones, A. M., A. Nguyen Dinh Cat, G. E. Callera, A. Yogi, D. Burger, Y. He, J. W. Corrêa, A. M. Gagnon, C. E. Gomez-Sanchez, E. P. Gomez-Sanchez, A. Sorisky, T. C. Ooi, M. Ruzicka, K. D. Burns & R. M. Touyz (2012) Adipocytes produce aldosterone through calcineurin-dependent signaling pathways: implications in diabetes mellitus-associated obesity and vascular dysfunction. *Hypertension,* 59**,** 1069-78.

Cabassi, A., P. Coghi, P. Govoni, E. Barouhiel, E. Speroni, S. Cavazzini, A. M. Cantoni, R. Scandroglio & E. Fiaccadori (2005) Sympathetic modulation by carvedilol and losartan reduces angiotensin II-mediated lipolysis in subcutaneous and visceral fat. *J Clin Endocrinol Metab,* 90**,** 2888-97.

Calhoun, D. A. & K. Sharma (2010) The role of aldosteronism in causing obesity-related cardiovascular risk. *Cardiol Clin,* 28**,** 517-27.

Cannon, B. & J. Nedergaard (2004) Brown adipose tissue: function and physiological significance. *Physiol Rev,* 84**,** 277-359.

Caprio, M., B. G. Newfell, A. la Sala, W. Baur, A. Fabbri, G. Rosano, M. E. Mendelsohn & I. Z. Jaffe (2008) Functional mineralocorticoid receptors in human vascular endothelial cells regulate intercellular adhesion molecule-1 expression and promote leukocyte adhesion. *Circ Res,* 102**,** 1359-67.

Carobbio, S., V. Pellegrinelli & A. Vidal-Puig (2017) Adipose Tissue Function and Expandability as Determinants of Lipotoxicity and the Metabolic Syndrome. *Adv Exp Med Biol,* 960**,** 161-196.

Charmandari, E., C. Tsigos & G. Chrousos (2005) Endocrinology of the stress response. *Annu Rev Physiol,* 67**,** 259-84.

Chaves, V. E., D. Frasson & N. H. Kawashita (2011) Several agents and pathways regulate lipolysis in adipocytes. *Biochimie,* 93**,** 1631-40.

Chen, Y. D., B. B. Varasteh & G. M. Reaven (1993) Plasma lactate concentration in obesity and type 2 diabetes. *Diabete Metab,* 19**,** 348-54.

Conceicao-Vertamatti, A. G., F. Borghi, F. Canova & D. M. Grassi-Kassisse (2017) History of vascular reactivity models and their involvement in hypertension pathogenesis. *Vasa***,** 1-9.

Cong, J., P. Li, L. Zheng & J. Tan (2016) Prevalence and Risk Factors of Infertility at a Rural Site of Northern China. *PLoS One,* 11**,** e0155563.

Consoli, A., N. Nurjhan & J. Gerich (1989) Rates of appearance and disappearance of plasma lactate after oral glucose: implications for indirect-pathway hepatic glycogen repletion in man. *Clin Physiol Biochem,* 7**,** 70-8.

Cook, K. S., H. Y. Min, D. Johnson, R. J. Chaplinsky, J. S. Flier, C. R. Hunt & B. M. Spiegelman (1987) Adipsin: a circulating serine protease homolog secreted by adipose tissue and sciatic nerve. *Science,* 237**,** 402-5.

Crandall, D. L., F. M. Lai, F. J. Huggins, T. K. Tanikella & P. Cervoni (1983) Effect of caloric restriction on cardiac reactivity and beta-adrenoceptor concentration. *Am J Physiol,* 244**,** H444-8.

Crege, D. R. X. d. O., H. J. V. Silveira, É. A. Chaim, J. C. Pareja, A. Géloen & D. M. Grassi-Kassisse. 2014. Sex Difference in Lactate Production by Adipocytes from Lean Humans. Open Journal of Endocrine and Metabolic Diseases.

Dall'Asta, C., P. Vedani, P. Manunta, P. Pizzocri, M. Marchi, M. Paganelli, F. Folli & A. E. Pontiroli (2009) Effect of weight loss through laparoscopic gastric banding on blood pressure, plasma renin activity and aldosterone levels in morbid obesity. *Nutr Metab Cardiovasc Dis,* 19**,** 110-4.

Dias, S., S. Paredes & L. Ribeiro (2018) Drugs Involved in Dyslipidemia and Obesity Treatment: Focus on Adipose Tissue. *Int J Endocrinol,* 2018**,** 2637418.

DiGirolamo, M., F. D. Newby & J. Lovejoy (1992) Lactate production in adipose tissue: a regulated function with extra-adipose implications. *FASEB J,* 6**,** 2405-12.

Dornas, W. C. & M. E. Silva (2011) Animal models for the study of arterial hypertension. *J Biosci,* 36**,** 731-7.

Duncan, R. E., M. Ahmadian, K. Jaworski, E. Sarkadi-Nagy & H. S. Sul (2007) Regulation of lipolysis in adipocytes. *Annu Rev Nutr,* 27**,** 79-101.

Ehrhart-Bornstein, M., K. Arakelyan, A. W. Krug, W. A. Scherbaum & S. R. Bornstein (2004) Fat cells may be the obesity-hypertension link: human adipogenic factors stimulate aldosterone secretion from adrenocortical cells. *Endocr Res,* 30**,** 865-70.

El Ghorayeb, N., I. Bourdeau & A. Lacroix (2016) Role of ACTH and Other Hormones in the Regulation of Aldosterone Production in Primary Aldosteronism. *Front Endocrinol (Lausanne),* 7**,** 72.

Engeli, S., P. Schling, K. Gorzelniak, M. Boschmann, J. Janke, G. Ailhaud, M. Teboul, F. Massiéra & A. M. Sharma (2003) The adipose-tissue renin-angiotensin-aldosterone system: role in the metabolic syndrome? *Int J Biochem Cell Biol,* 35**,** 807-25.

Engeli, S., R. Stinkens, T. Heise, M. May, G. H. Goossens, E. E. Blaak, B. Havekes, T. Jax, D. Albrecht, P. Pal, U. Tegtbur, S. Haufe, T. H. Langenickel & J. Jordan (2018) Effect of Sacubitril/Valsartan on Exercise-Induced Lipid Metabolism in Patients With Obesity and Hypertension. *Hypertension,* 71**,** 70-7.

Faintrenie, G. & A. Geloen (1996) Alpha-1 adrenergic regulation of lactate production by white adipocytes. *J Pharmacol Exp Ther,* 277**,** 235-8.

Farkas, G. J., A. S. Gorgey, D. R. Dolbow, A. S. Berg & D. R. Gater (2018) Sex dimorphism in the distribution of adipose tissue and its influence on proinflammatory adipokines and cardiometabolic profiles in motor complete spinal cord injury. *J Spinal Cord Med***,** 1-15.

Farquharson, C. A. & A. D. Struthers (2002) Aldosterone induces acute endothelial dysfunction in vivo in humans: evidence for an aldosterone-induced vasculopathy. *Clin Sci (Lond),* 103**,** 425-31.

Faulkner, J. L., T. Bruder-Nascimento & E. J. Belin de Chantemèle (2018) The regulation of aldosterone secretion by leptin: implications in obesity-related cardiovascular disease. *Curr Opin Nephrol Hypertens,* 27**,** 63-69.

Feldmann, H. M., V. Golozoubova, B. Cannon & J. Nedergaard (2009) UCP1 ablation induces obesity and abolishes diet-induced thermogenesis in mice exempt from thermal stress by living at thermoneutrality. *Cell Metab,* 9**,** 203-9.

Fruhbeck, G., L. Mendez-Gimenez, J. A. Fernandez-Formoso, S. Fernandez & A. Rodriguez (2014) Regulation of adipocyte lipolysis. *Nutr Res Rev,* 27**,** 63-93.

Garg, A. (2004) Acquired and inherited lipodystrophies. *N Engl J Med,* 350**,** 1220-34.

Goldstein, D. S. (2003) Catecholamines and stress. *Endocr Regul,* 37**,** 69-80.

Goossens, G. H., E. E. Blaak, P. Arner, W. H. Saris & M. A. van Baak (2007) Angiotensin II: a hormone that affects lipid metabolism in adipose tissue. *Int J Obes (Lond),* 31**,** 382-4.

Goossens, G. H., E. E. Blaak, W. H. Saris & M. A. van Baak (2004) Angiotensin II-induced effects on adipose and skeletal muscle tissue blood flow and

lipolysis in normal-weight and obese subjects. *J Clin Endocrinol Metab,* 89**,** 2690-6.

Gorden, P. & O. Gavrilova (2003) The clinical uses of leptin. *Curr Opin Pharmacol,* 3**,** 655-9.

Harrap, S. B., W. M. Van der Merwe, S. A. Griffin, F. Macpherson & A. F. Lever (1990) Brief angiotensin converting enzyme inhibitor treatment in young spontaneously hypertensive rats reduces blood pressure long-term. *Hypertension,* 16**,** 603-14.

Heden, T. D., Y. Liu & J. A. Kanaley (2017) Exercise timing and blood lactate concentrations in individuals with type 2 diabetes. *Appl Physiol Nutr Metab,* 42**,** 732-737.

Herbst, K. L. (2012) Rare adipose disorders (RADs) masquerading as obesity. *Acta Pharmacol Sin,* 33**,** 155-72.

Herranz, P., R. de Lucas, L. Pérez-España & M. Mayor (2008) Lipodystrophy syndromes. *Dermatol Clin,* 26**,** 569-78, ix.

Hill, B. G. (2015) Insights into an adipocyte whitening program. *Adipocyte,* 4**,** 75-80.

Hu, E., P. Liang & B. M. Spiegelman (1996) AdipoQ is a novel adipose-specific gene dysregulated in obesity. *J Biol Chem,* 271**,** 10697-703.

Hökfelt, B. (1978) Sympathetic amines, renin and blood pressure. *Acta Endocrinol Suppl (Copenh),* 216**,** 67-74.

Iams, S. G., J. P. McMurthy & B. C. Wexler (1979) Aldosterone, deoxycorticosterone, corticosterone, and prolactin changes during the lifespan of chronically and spontaneously hypertensive rats. *Endocrinology,* 104**,** 1357-63.

Ikeda, N., D. Sapienza, R. Guerrero, W. Aekplakorn, M. Naghavi, A. H. Mokdad, R. Lozano, C. J. Murray & S. S. Lim (2014) Control of hypertension with medication: a comparative analysis of national surveys in 20 countries. *Bull World Health Organ,* 92**,** 10-19c.

Infante, M., A. Armani, C. Mammi, A. Fabbri & M. Caprio (2017) Impact of Adrenal Steroids on Regulation of Adipose Tissue. *Compr Physiol,* 7**,** 1425-1447.

Jansson, P. A., A. Larsson, U. Smith & P. Lönnroth (1994) Lactate release from the subcutaneous tissue in lean and obese men. *J Clin Invest,* 93**,** 240-6.

Judy, W. V., A. M. Watanabe, D. P. Henry, H. R. Besch & B. Aprison (1978) Effect of l-dopa on sympathetic nerve activity and blood pressure in the spontaneously hypertensive rat. *Circ Res,* 43**,** 24-8.

Kawarazaki, W. & T. Fujita (2016) The Role of Aldosterone in Obesity-Related Hypertension. *Am J Hypertens,* 29**,** 415-23.

Klimas, J., V. Vaja, M. Vercinska, J. Kyselovic & P. Krenek (2012) Discrepant regulation of QT (QTc) interval duration by calcium channel blockade and angiotensin converting enzyme inhibition in experimental hypertension. *Basic Clin Pharmacol Toxicol,* 111**,** 279-88.

Kristek, F., M. Malekova & S. Cacanyiova (2013) Long-term effect of prazosin and losartan administration on blood pressure, heart, carotid artery, and acetylcholine induced dilation of cardiovascular system of young Wistar rats and SHR. *Gen Physiol Biophys,* 32**,** 235-43.

Kvetnansky, R., R. McCarty, N. B. Thoa, C. R. Lake & I. J. Kopin (1979) Sympatho-adrenal responses of spontaneously hypertensive rats to immobilization stress. *Am J Physiol,* 236**,** H457-62.

Lamounier-Zepter, V., M. Ehrhart-Bornstein & S. R. Bornstein (2005) Mineralocorticoid-stimulating activity of adipose tissue. *Best Pract Res Clin Endocrinol Metab,* 19**,** 567-75.

Langin, D. (2006) Adipose tissue lipolysis as a metabolic pathway to define pharmacological strategies against obesity and the metabolic syndrome. *Pharmacol Res.,* 53**,** 482-91.

--- (2010) Adipose tissue lipolysis revisited (again!): lactate involvement in insulin antilipolytic action. *Cell Metab,* 11**,** 242-3.

Lee, M. J. (2018) Transforming growth factor beta superfamily regulation of adipose tissue biology in obesity. *Biochim Biophys Acta,* 1864**,** 1160-1171.

Lee, M. J., Y. Wu & S. K. Fried (2013) Adipose tissue heterogeneity: implication of depot differences in adipose tissue for obesity complications. *Mol Aspects Med,* 34**,** 1-11.

Lelliott, C. & A. J. Vidal-Puig (2004) Lipotoxicity, an imbalance between lipogenesis de novo and fatty acid oxidation. *Int J Obes Relat Metab Disord,* 28 Suppl 4**,** S22-8.

Leong, X. F., C. Y. Ng & K. Jaarin (2015) Animal Models in Cardiovascular Research: Hypertension and Atherosclerosis. *Biomed Res Int,* 2015**,** 528757.

Lezama-Martínez, D., I. Valencia-Hernández, J. Flores-Monroy & L. Martínez-Aguilar (2017) Combination of β Adrenergic Receptor Block and Renin-Angiotensin System Inhibition Diminished the Angiotensin II-Induced Vasoconstriction and Increased Bradykinin-Induced Vasodilation in Hypertension. *Dose Response,* 15**,** 1559325817737932.

Li, Y., V. Periwal, S. W. Cushman & K. G. Stenkula (2016) Adipose cell hypertrophy precedes the appearance of small adipocytes by 3 days in C57BL/6 mouse upon changing to a high fat diet. *Adipocyte,* 5**,** 81-7.

Lin, Y., M. W. Rajala, J. P. Berger, D. E. Moller, N. Barzilai & P. E. Scherer (2001) Hyperglycemia-induced production of acute phase reactants in adipose tissue. *J Biol Chem,* 276**,** 42077-83.

Lovejoy, J., B. Mellen & M. Digirolamo (1990) Lactate generation following glucose ingestion: relation to obesity, carbohydrate tolerance and insulin sensitivity. *Int J Obes,* 14**,** 843-55.

Lovejoy, J., F. D. Newby, S. S. Gebhart & M. DiGirolamo (1992) Insulin resistance in obesity is associated with elevated basal lactate levels and diminished lactate appearance following intravenous glucose and insulin. *Metabolism,* 41**,** 22-7.

Lowell, B. B., V. S-Susulic, A. Hamann, J. A. Lawitts, J. Himms-Hagen, B. B. Boyer, L. P. Kozak & J. S. Flier (1993) Development of obesity in transgenic mice after genetic ablation of brown adipose tissue. *Nature,* 366**,** 740-2.

Ma, X., P. Lee, D. J. Chisholm & D. E. James (2015) Control of adipocyte differentiation in different fat depots; implications for pathophysiology or therapy. *Front Endocrinol (Lausanne),* 6**,** 1.

MacLean, P. S., J. A. Higgins, E. D. Giles, V. D. Sherk & M. R. Jackman (2015) The role for adipose tissue in weight regain after weight loss. *Obes Rev,* 16 Suppl 1**,** 45-54.

Maeda, K., K. Okubo, I. Shimomura, T. Funahashi, Y. Matsuzawa & K. Matsubara (1996) cDNA cloning and expression of a novel adipose specific collagen-

like factor, apM1 (AdiPose Most abundant Gene transcript 1). *Biochem Biophys Res Commun,* 221**,** 286-9.

Marlatt, K. L. & E. Ravussin (2017) Brown Adipose Tissue: an Update on Recent Findings. - PubMed - NCBI. *Current Obesity Reports*.

Marzolla, V., A. Armani, M. C. Zennaro, F. Cinti, C. Mammi, A. Fabbri, G. M. Rosano & M. Caprio (2012) The role of the mineralocorticoid receptor in adipocyte biology and fat metabolism. *Mol Cell Endocrinol,* 350**,** 281-8.

McManus, R. J., J. Mant, M. Franssen, A. Nickless, C. Schwartz, J. Hodgkinson, P. Bradburn, A. Farmer, S. Grant, S. M. Greenfield, C. Heneghan, S. Jowett, U. Martin, S. Milner, M. Monahan, S. Mort, E. Ogburn, R. Perera-Salazar, S. A. Shah, L. M. Yu, L. Tarassenko & F. D. R. Hobbs (2018) Efficacy of self-monitored blood pressure, with or without telemonitoring, for titration of antihypertensive medication (TASMINH4): an unmasked randomised controlled trial. *Lancet,* 391**,** 949-959.

McNamara, J. P. & K. Huber (2018) Metabolic and Endocrine Role of Adipose Tissue During Lactation. *Annu Rev Anim Biosci,* 6**,** 177-195.

Moitra, J., M. M. Mason, M. Olive, D. Krylov, O. Gavrilova, B. Marcus-Samuels, L. Feigenbaum, E. Lee, T. Aoyama, M. Eckhaus, M. L. Reitman & C. Vinson (1998) Life without white fat: a transgenic mouse. *Genes Dev,* 12**,** 3168-81.

Murawska-Ciałowicz, E. (2017) Adipose tissue - morphological and biochemical characteristic of different depots. *Postepy Hig Med Dosw (Online),* 71**,** 466-484.

Nagase, M., S. Yoshida, S. Shibata, T. Nagase, T. Gotoda, K. Ando & T. Fujita (2006) Enhanced aldosterone signaling in the early nephropathy of rats with metabolic syndrome: possible contribution of fat-derived factors. *J Am Soc Nephrol,* 17**,** 3438-46.

Nakano, Y., T. Tobe, N. H. Choi-Miura, T. Mazda & M. Tomita (1996) Isolation and characterization of GBP28, a novel gelatin-binding protein purified from human plasma. *J Biochem,* 120**,** 803-12.

Newby, F. D., F. Bayo, S. V. Thacker, M. Sykes & M. DiGirolamo (1989) Effects of streptozocin-induced diabetes on glucose metabolism and lactate

release by isolated fat cells from young lean and older, moderately obese rats. *Diabetes,* 38**,** 237-43.

Okamoto, K. & K. Aoki (1963) Development of a strain of spontaneously hypertensive rats. *Jpn Circ J,* 27**,** 282-93.

Okla, M., J. Kim, K. Koehler & S. Chung (2017) Dietary Factors Promoting Brown and Beige Fat Development and Thermogenesis. *Adv Nutr,* 8**,** 473-483.

Pahlavani, M., N. S. Kalupahana, L. Ramalingam & N. Moustaid-Moussa (2017) Regulation and Functions of the Renin-Angiotensin System in White and Brown Adipose Tissue. *Compr Physiol,* 7**,** 1137-1150.

Pajvani, U. B., M. E. Trujillo, T. P. Combs, P. Iyengar, L. Jelicks, K. A. Roth, R. N. Kitsis & P. E. Scherer (2005) Fat apoptosis through targeted activation of caspase 8: a new mouse model of inducible and reversible lipoatrophy. *Nat Med,* 11**,** 797-803.

Palmer, A. K. & J. L. Kirkland (2016) Aging and adipose tissue: potential interventions for diabetes and regenerative medicine. *Exp Gerontol,* 86**,** 97-105.

Paulis, L. & T. Unger (2010) Novel therapeutic targets for hypertension. *Nat Rev Cardiol,* 7**,** 431-41.

Peirce, V., S. Carobbio & A. Vidal-Puig (2014) The different shades of fat. *Nature,* 510**,** 76-83.

Pellegrinelli, V., S. Carobbio & A. Vidal-Puig (2016) Adipose tissue plasticity: how fat depots respond differently to pathophysiological cues. *Diabetologia,* 59**,** 1075-88.

Perk, J., G. De Backer, H. Gohlke, I. Graham, Z. Reiner, M. Verschuren, C. Albus, P. Benlian, G. Boysen, R. Cifkova, C. Deaton, S. Ebrahim, M. Fisher, G. Germano, R. Hobbs, A. Hoes, S. Karadeniz, A. Mezzani, E. Prescott, L. Ryden, M. Scherer, M. Syvänne, W. J. Scholte op Reimer, C. Vrints, D. Wood, J. L. Zamorano, F. Zannad, E. A. f. C. P. R. (EACPR) & E. C. f. P. G. (CPG) (2012) European Guidelines on cardiovascular disease prevention in clinical practice (version 2012). The Fifth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by

representatives of nine societies and by invited experts). *Eur Heart J,* 33**,** 1635-701.

Picon, R. V., J. S. Dias-da-Costa, F. D. Fuchs, M. T. A. Olinto, N. K. Choudhry & S. C. Fuchs (2017) Hypertension Management in Brazil: Usual Practice in Primary Care—A Meta-Analysis. *International Journal of Hypertension***,** 9.

Picon, R. V., F. D. Fuchs, L. B. Moreira, G. Riegel & S. C. Fuchs (2012) Trends in prevalence of hypertension in Brazil: a systematic review with meta-analysis. *PLoS One,* 7**,** e48255.

Pravenec, M., V. Křen, V. Landa, P. Mlejnek, A. Musilová, J. Šilhavý, M. Šimáková & V. Zídek (2014) Recent progress in the genetics of spontaneously hypertensive rats. *Physiol Res,* 63 Suppl 1**,** S1-8.

Rafacho, A., L. M. Goncalves-Neto, F. B. Ferreira, A. O. Protzek, A. C. Boschero, E. A. Nunes & D. B. Zoccal (2013) Glucose homoeostasis in rats exposed to acute intermittent hypoxia. *Acta Physiol (Oxf),* 209**,** 77-89.

Regan, C. P., S. P. Bishop & K. H. Berecek (1997) Early, short-term treatment with captopril permanently attenuates cardiovascular changes in spontaneously hypertensive rats. *Clin Exp Hypertens,* 19**,** 1161-77.

Ross, S. R., R. A. Graves & B. M. Spiegelman (1993) Targeted expression of a toxin gene to adipose tissue: transgenic mice resistant to obesity. *Genes Dev,* 7**,** 1318-24.

Rothwell, N. J. & M. J. Stock (1983) Luxuskonsumption, diet-induced thermogenesis and brown fat: the case in favour. *Clin Sci (Lond),* 64**,** 19-23.

Rutkowski, J. M., J. H. Stern & P. E. Scherer (2015) The cell biology of fat expansion. *J Cell Biol.,* 208**,** 501-12.

Rydén, M. & P. Arner (2017) Subcutaneous Adipocyte Lipolysis Contributes to Circulating Lipid Levels. *Arterioscler Thromb Vasc Biol,* 37**,** 1782-7.

Sanchez-Gurmaches, J. & D. A. Guertin (2014) Adipocyte lineages: tracing back the origins of fat. *Biochim Biophys Acta,* 1842**,** 340-51.

Sapolsky, R. M., L. M. Romero & A. U. Munck (2000) How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocr Rev,* 21**,** 55-89.

Scherer, P. E. (2006) Adipose tissue: from lipid storage compartment to endocrine organ. *Diabetes,* 55**,** 1537-45.

Scherer, P. E., S. Williams, M. Fogliano, G. Baldini & H. F. Lodish (1995) A novel serum protein similar to C1q, produced exclusively in adipocytes. *J Biol Chem,* 270**,** 26746-9.

Schinner, S., H. S. Willenberg, D. Krause, M. Schott, V. Lamounier-Zepter, A. W. Krug, M. Ehrhart-Bornstein, S. R. Bornstein & W. A. Scherbaum (2007) Adipocyte-derived products induce the transcription of the StAR promoter and stimulate aldosterone and cortisol secretion from adrenocortical cells through the Wnt-signaling pathway. *Int J Obes (Lond),* 31**,** 864-70.

Schoettl, T., I. P. Fischer & S. Ussar (2018) Heterogeneity of adipose tissue in development and metabolic function. *J Exp Biol,* 221.

Scott, K. A., S. J. Melhorn & R. R. Sakai (2012) Effects of Chronic Social Stress on Obesity. *Curr Obes Rep,* 1**,** 16-25.

Sellayah, D. & D. Sikder (2014) Orexin restores aging-related brown adipose tissue dysfunction in male mice. *Endocrinology,* 155**,** 485-501.

Sepa-Kishi, D. M. & R. B. Ceddia (2018) White and beige adipocytes: are they metabolically distinct? *Horm Mol Biol Clin Investig*.

Sevá Pessôa, B., N. van der Lubbe, K. Verdonk, A. J. Roks, E. J. Hoorn & A. H. Danser (2013) Key developments in renin-angiotensin-aldosterone system inhibition. *Nat Rev Nephrol,* 9**,** 26-36.

Shimomura, I., R. E. Hammer, J. A. Richardson, S. Ikemoto, Y. Bashmakov, J. L. Goldstein & M. S. Brown (1998) Insulin resistance and diabetes mellitus in transgenic mice expressing nuclear SREBP-1c in adipose tissue: model for congenital generalized lipodystrophy. *Genes Dev,* 12**,** 3182-94.

Shum, M., S. Pinard, M. O. Guimond, S. M. Labbé, C. Roberge, J. P. Baillargeon, M. F. Langlois, M. Alterman, C. Wallinder, A. Hallberg, A. C. Carpentier & N. Gallo-Payet (2013) Angiotensin II type 2 receptor promotes adipocyte differentiation and restores adipocyte size in high-fat/high-fructose diet-induced insulin resistance in rats. *Am J Physiol Endocrinol Metab,* 304**,** E197-210.

Sowers, J., M. Tuck, N. D. Asp & E. Sollars (1981) Plasma aldosterone and corticosterone responses to adrenocorticotropin, angiotensin, potassium,

and stress in spontaneously hypertensive rats. *Endocrinology,* 108**,** 1216-21.

Sowers, J. R., E. G. Sollars, M. L. Tuck & N. D. Asp (1980) Dopaminergic Modulation of renin activity and aldosterone and prolactin secretion in the spontaneously hypertensive rat. *Proc Soc Exp Biol Med,* 164**,** 598-603.

Steptoe, A. (2000) Stress, social support and cardiovascular activity over the working day. *Int J Psychophysiol,* 37**,** 299-308.

Szczepanska-Sadowska, E., K. Czarzasta & A. Cudnoch-Jedrzejewska (2018) Dysregulation of the Renin-Angiotensin System and the Vasopressinergic System Interactions in Cardiovascular Disorders. *Curr Hypertens Rep,* 20**,** 19.

Tchkonia, T., D. E. Morbeck, T. von Zglinicki, J. van Deursen, J. Lustgarten, H. Scrable, S. Khosla, M. D. Jensen & J. L. Kirkland (2010) Fat tissue, aging, and cellular senescence. *Aging Cell,* 9**,** 667-84.

van der Stelt, I., F. Hoevenaars, J. Siroka, L. de Ronde, D. Friedecky, J. Keijer & E. van Schothorst (2017) Metabolic Response of Visceral White Adipose Tissue of Obese Mice Exposed for 5 Days to Human Room Temperature Compared to Mouse Thermoneutrality. *Front Physiol,* 8**,** 179.

Vernochet, C., F. Damilano, A. Mourier, O. Bezy, M. A. Mori, G. Smyth, A. Rosenzweig, N. G. Larsson & C. R. Kahn (2014) Adipose tissue mitochondrial dysfunction triggers a lipodystrophic syndrome with insulin resistance, hepatosteatosis, and cardiovascular complications. *FASEB J,* 28**,** 4408-19.

Vicennati, V., S. Garelli, E. Rinaldi, G. Di Dalmazi, U. Pagotto & R. Pasquali (2014) Cross-talk between adipose tissue and the HPA axis in obesity and overt hypercortisolemic states. *Horm Mol Biol Clin Investig,* 17**,** 63-77.

Wang, S., Y. Li, W. Miao, H. Zhao, F. Zhang, N. Liu, G. Su & X. Cai (2016) Angiopoietin-like protein 2 expression is suppressed by angiotensin II via the angiotensin II type 1 receptor in rat cardiomyocytes. *Mol Med Rep*.

Winkler, M., M. Bader, F. Schuster, I. Stölting, S. Binder & W. Raasch (2018) Development of obesity can be prevented in rats by chronic icv infusions of AngII but less by Ang(1-7). *Pflugers Arch*.

Wu, C. H., S. Mohammadmoradi, J. Thompson, W. Su, M. Gong, G. Nguyen & F. Yiannikouris (2016) Adipocyte (Pro)Renin-Receptor Deficiency Induces Lipodystrophy, Liver Steatosis and Increases Blood Pressure in Male Mice. *Hypertension,* 68**,** 213-9.

Yvan-Charvet, L., P. Even, M. Bloch-Faure, M. Guerre-Millo, N. Moustaid-Moussa, P. Ferre & A. Quignard-Boulange (2005) Deletion of the angiotensin type 2 receptor (AT2R) reduces adipose cell size and protects from diet-induced obesity and insulin resistance. *Diabetes,* 54**,** 991-9.

Zhang, Y., R. Proenca, M. Maffei, M. Barone, L. Leopold & J. M. Friedman (1994) Positional cloning of the mouse obese gene and its human homologue. *Nature,* 372**,** 425-32.

Zhu, Q. & P. E. Scherer (2018) Immunologic and endocrine functions of adipose tissue: implications for kidney disease. *Nat Rev Nephrol,* 14**,** 105-120.

Zorad, S., J. T. Dou, J. Benicky, D. Hutanu, K. Tybitanclova, J. Zhou & J. M. Saavedra (2006) Long-term angiotensin II AT1 receptor inhibition produces adipose tissue hypotrophy accompanied by increased expression of adiponectin and PPARgamma. *Eur J Pharmacol,* 552**,** 112-22.

ANEXOS

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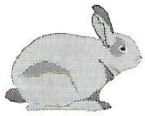

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Sexo: machos
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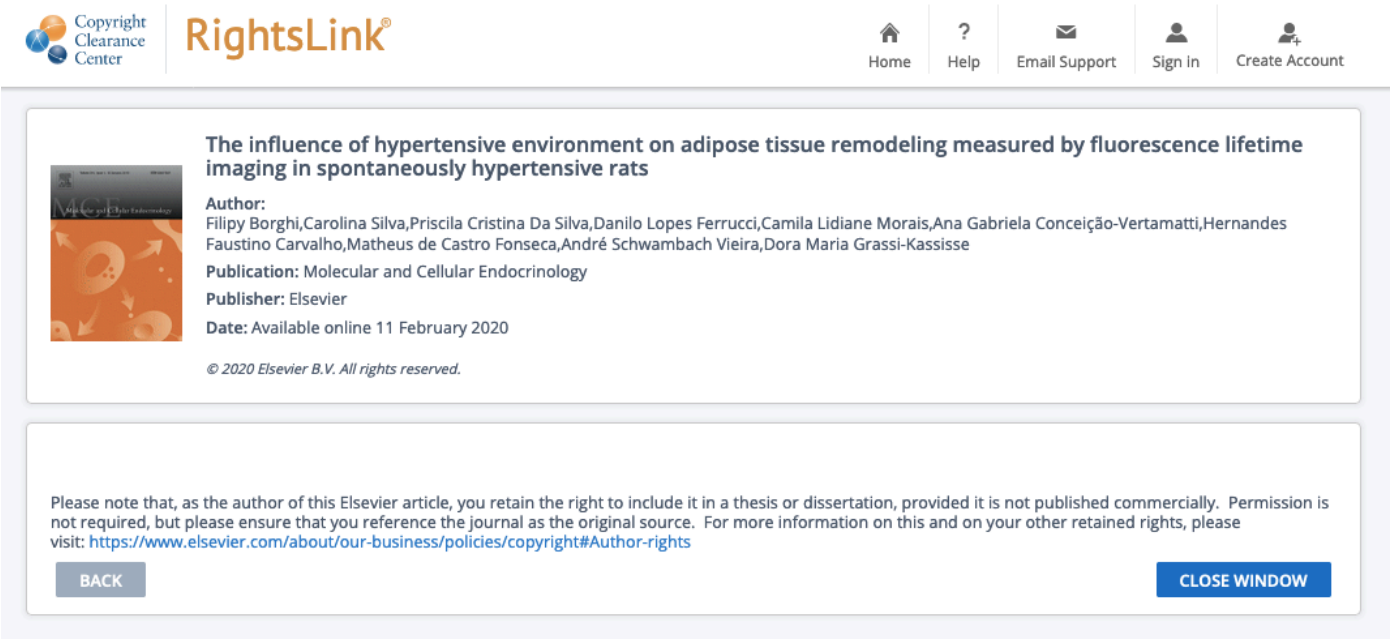

ARTIGOS

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REVIEW

The adipose tissue and the involvement of the renin–angiotensin–aldosterone system in cardiometabolic syndrome

F. Borghi[1] · **B. Sevá-Pessôa**[2] · **D. M. Grassi-Kassisse**[1]

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Abstract Cardiometabolic diseases are linked to a cluster of modifiable factors, including risk factors closely related to central adiposity. Chronic renin–angiotensin–aldosterone system (RAAS) activation has far-reaching effects on cardiometabolic risk and is a substantial contributor to this clinical condition. RAAS components are locally expressed in the vessels and adipose tissue. This review appoints RAAS, through the classical and the alternative view, as the main mediator of the cross-talk in cardiometabolic syndrome.

Keywords Adipose tissue · Angiotensin · Cardiometabolic syndrome · Renin–angiotensin–aldosterone system · Adiposity

Introduction

Cardiometabolic diseases are linked to a cluster of modifiable factors, including established risk factors such as obesity, insulin resistance, dyslipidemias, hypertension and other risk factors closely related to central adiposity (Matfin 2007). Obesity and insulin resistance are predisposing factors for cardiovascular diseases (Jacome-Sosa et al. 2016), which are the main cause of death worldwide, with the global burden of disease being led by hypertension (Perk et al. 2012). Additionally, chronic renin–angiotensin–aldosterone system (RAAS) activation has far-reaching effects on cardiometabolic risk besides its substantial contribution to cardiovascular disease and renal dysfunction (Epstein et al. 2012). This interaction could be a result of elevated levels of serum aldosterone observed in obesity (Engeli and Sharma 2001), which may be due to an as-yet unidentified factor from adipose tissue that results in increased synthesis of aldosterone (Whaley-Connell et al. 2007).

The RAAS is the most important regulator of renal and cardiovascular function and it plays a main role in the equilibrium of the cardiovascular system. The RAAS affects the cardiovascular system in two ways: directly, changing the cardiac morphology and vascular function, or indirectly, by maintaining blood volume and electrolytic balance, cardiac reflexes and stimulating hormones secretion, working in harmony with the sympathetic nervous system (Paulis and Unger 2010).

The RAAS classic pathway starts through pro-renin receptor stimulation induced by low blood pressure or sympathetic nerve impulses followed by activation of renin, which will lead to an enzymatic cascade, cleaving angiotensinogen and generating angiotensin I (Ang I). Ang I is the target of an angiotensin-converting enzyme (ACE) producing angiotensin II (Ang II), a potent vasoconstrictor agent. The introduction of RAAS blockers, such as ACE inhibitors, angiotensin II-type 1 (AT1)-receptor blockers (ARBs) and mineralocorticoid-receptor antagonists (MRAs) have contributed to the understanding of this system. However, most of the beneficial effects, for example as an antihypertensive agent, are due to blockade of the angiotensin II–AT1 receptor–aldosterone–mineralocorticoid receptor axis (Takahashi et al. 2011).

In the classical view of the RAAS, Ang II, in higher amounts, is the endogenous mediator responsible for many adverse cardiovascular effects such as hypertension,

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endothelial dysfunction and fibrosis. These effects are mainly due to the activation of the angiotensin II-type 1 receptor (AT1R). In addition, Ang II also stimulates aldosterone production, which in turn induces further increases in volemia and blood pressure due to water and salt retention (Seva Pessoa et al. 2013).

In the past 10 years, accumulated evidence has identified another angiotensin: angiotensin-(1–7) [Ang-(1–7)]. This angiotensin is produced by activity of ACE2, a homologue of ACE, in Ang II. Ang-(1–7) has its own receptor, called Mas, which when stimulated induces vasodilation and cardio-protection and inhibits angiogenesis and cell growth, while its effects are opposed to the pressor, proliferative, profibrotic and prothrombotic actions mediated by Ang II via AT1R (Gironacci 2015). This new RAAS pathway is called the alternative view of RAAS. RAAS is also implicated in impaired glucose homeostasis. Subjects on ACE inhibitors or ARBs have a lower risk of developing type 2 diabetes compared with individuals on other anti-hypertensive medications (Frigolet et al. 2013; Vermes et al. 2003). Improvements in insulin sensitivity and glucose metabolism have also been demonstrated in rats and humans that are treated with RAS antagonists (Frigolet et al. 2013; Nagel et al. 2006).

During these findings, evidence was found that the RAAS components are locally expressed in white adipose tissue (Marcus et al. 2013), harnessing this system to cardiometabolic syndrome. It is already well known that adipose tissue is an important and distinct endocrine organ. The production of adipokines is involved in the metabolic equilibrium but an imbalance in its production induces metabolic syndrome, type 2 diabetes, renal damage and cardiovascular diseases (Jing et al. 2013). In this review, we will describe the participation of RAAS, through the classical and the alternative view and suggest this system as the main mediator of the cross-talk in the cardiometabolic syndrome.

RAAS: Classical view

The classical renin–angiotensin–aldosterone hormonal cascade begins with the biosynthesis of renin by the juxtaglomerular (JG) cells. Renin is synthesized as a preprohormone and active renin is formed by proteolytic removal of a 43-amino-acid prosegment peptide from the N-terminus of prorenin, the proenzyme or renin precursor. Mature renin is stored in granules of the JG cells and is released by an exocytic process involving stimulus–secretion coupling into the renal and then systemic circulation. In addition to this regulated pathway, the kidney also releases unprocessed prorenin via a constitutive pathway. In fact, prorenin accounts for about 70–90 % of the immunoreactive renin in human circulation. The potential biological significance of this finding is currently poorly understood (Atlas 2007).

Active renin secretion is under the control of four interdependent factors:

1. A renal baroreceptor mechanism present in the afferent arteriole, which is sensitive to changes in renal perfusion pressure;
2. Changes in the amounts of sodium chloride delivery, sensed mainly as changes in Cl^- concentration, to the macula densa cells present in distal tubules;
3. Sympathetic nerve stimulation via beta-1 adrenergic receptors, and;
4. A negative feedback by a direct action of Ang II on the JG cells (Brown 2006).

Renin secretion is stimulated by a fall in perfusion pressure or in NaCl delivery and by an increase in sympathetic activity. However, renin is also synthesized in other tissues, including brain, adrenal gland, ovary and visceral adipose tissue, even in heart and vascular tissue. The factors regulating synthesis and possible actions of renin in these other tissues are poorly understood (Atlas 2007).

The active renin cleaves the angiotensinogen to Ang I, which in turn is converted to Ang II by ACE (Carey and Siragy 2003). The Ang II actions are mainly mediated by the AT1 receptor, besides establishing the control of reno-cardiovascular equilibrium, promoting the constriction of renal and systemic arterioles and also stimulating the production of aldosterone from the adrenal cortex, which is involved with electrolytic balance, inducing reabsorption of sodium and consequently water and hydrogen secretion, mainly in proximal segments of the nephron (Atlas 2007; Seva Pessoa et al. 2013).

Angiotensin-converting enzyme (ACE) inhibitors and the AT1R blocker are the major targets for cardioprotective therapies. Their beneficial effects are attributed to inhibition of the undesired AT1R stimulation and subsequent reduction in vascular tone, blood pressure, aldosterone, vasopressin and catecholamines release, inhibition of inflammation and attenuation of cell growth (Unger et al. 2011). These effects can be obtained not only by these blockades but perhaps also by stimulating or evidence of other pathways that have just been covered up by AT1 activity.

Considering inhibition therapy to elucidate the system's mechanisms, obviously renin is the most logical therapeutic target due to this enzyme being responsible for activation of all RAAS cascades (Atlas 2007). The renin inhibitors are indeed associated with the decrease in Ang I, Ang II and aldosterone levels and with reduction in blood pressure (Unger et al. 2011). The possible benefit in renin inhibition might be the attenuation of plasma renin activity, which is increased by ACE inhibition or AT1R blockade (Ménard et al. 1997). On the other hand, the high renin levels after

treatment with the renin blocker might escape from the renin inhibition and may not prevent binding with the prorenin receptor (Unger et al. 2011).

ACE inhibition prevents the conversion of Ang I to Ang II, reducing the Ang II levels and indirectly the aldosterone and vasopressin levels and additionally contributes to decreasing the sympathetic activity (Atlas 2007). ACE inhibition increases insulin sensitivity, which is particularly relevant in adipocytes, where ACE inhibition causes bradykinin accumulation (Frigolet et al. 2013; McCarty 2003), which increases glucose uptake in rat adipocytes via endothelial nitric oxide synthase-mediated JNK inhibition, which is a negative regulator of insulin signaling (Beard et al. 2006).

At the beginning of the 1990s, another pharmacotherapy was proposed as being responsible for decreasing the effects of RAAS. A selective AT1 receptor blocker (ARB) was shown to be a more effective blockade, since it blocks the interaction of Ang II to the receptor, regardless of where it was synthesized (Ferrario 2006). Against the ACE blockade, ARB therapy increases Ang II levels, which might stimulate the activation of type 2 angiotensin II (AT2) receptors, which are thought to oppose the effects of AT1 receptor activation, including vasoconstriction, stimulation of growth and remodeling, sympathetic nervous system activation and sodium and water retention (Seva Pessoa et al. 2013). These treatments also result in the production of high levels of Ang II metabolites, most importantly Ang-(1–7) (Seva Pessoa et al. 2013), which plays a key role in the alternative pathway, the *good axis* and PPARγ, which enhances insulin action by increasing the secretion of anti-inflammatory adipokine adiponectin and preadipocyte differentiation (Frigolet et al. 2013; Kintscher and Law 2005). Ang II synthesis may also be stimulated by the adrenocorticotrophic hormone (ACTH; corticotrophin), norepinephrine, endothelin and serotonin and inhibited by atrial natriuretic peptide and nitric oxide (NO) (Atlas 2007).

Against the effects of AT1R as a strong vasoconstrictor, AT2R is known as a vasodepressor element of RAAS (Sampson et al. 2012). Some studies have indicated that the hypotensive effect of AT1R blockade is attributed to AT2R stimulation (Carey et al. 2001; Maleki and Nematbakhsh 2016). Siragy and Carey (1997) demonstrated that AT2 receptors mediate cGMP4 through the generation of renal NO, which may explain its vasodilatory action. However, the physiological actions of Ang II at AT2 receptors have been difficult to elicit, at least in part because AT2 receptors have a low degree of expression compared with that of AT1 receptors (Carey et al. 2001). The Ang II responsiveness occurs due to the receptor presence in the tissue, thus AT2 activity is often masked by AT1 activity. Thereby, AT2 activity can be evidenced when the AT1 receptors are under blockade.

Ang II acting via the AT1 receptor is able to stimulate aldosterone production in adrenocortical cells and is the major regulator of sodium production and potassium balance (Atlas 2007). Aldosterone enhances the reabsorption of sodium and water in the distal tubules and collecting ducts during hypovolemia and thereby promotes potassium excretion during hyperkalemia (Atlas 2007; Funder 2002; Seva Pessoa et al. 2013). In hypovolemia, Ang II and aldosterone levels are increased, causing a synergistic activation of the sodium chloride cotransporter (NCC) and the epithelial sodium channel (ENaC), promoting maximal sodium reabsorption (Seva Pessoa et al. 2013). Meanwhile, potassium levels remain stable because the sodium reabsorption is electroneutral and does not require potassium secretion through the renal outer medullary potassium channel (ROMK), which is inhibited by angiotensin II (Seva Pessoa et al. 2013; Wei et al. 2007; Yue et al. 2011). Unlike hypovolemia, during the hyperkalemia, aldosterone levels are maximally increased, whereas Ang II levels are suppressed, resulting in NCC inhibition and increased sodium delivery to ENaCs. Maximum ENaC activity facilitates electrochemical sodium reabsorption, which promotes kaliuresis through the ROMK (Seva Pessoa et al. 2013).

RAAS: Alternative view

The alternative pathway consists in the stimulation of the G-protein-coupled Mas receptor by Ang-(1–7), which can be formed directly from Ang II by ACE2 but also from Ang I (Durik et al. 2012). The activation of the Mas receptor on the endothelial cells leads to the activation of endothelial NO synthase (van Twist et al. 2014). The NO produced induces relaxation of the vascular smooth muscle and consequently vasodilation. Furthermore, the stimulation of the Mas receptor counter regulates the effects of Ang II by inhibiting the intracellular pathways such as extracellular signal-regulated kinases 1/2 and c-Src that are induced by AT1R stimulation (van Twist et al. 2014; Verano-Braga et al. 2012). Thereby, the Ang-(1–7) induces vasorelaxation effects by two different pathways, first through NO-mediated vasodilation and also by inhibition of the Ang II vasoconstrictor effects (van Twist et al. 2014). In this way, Ang-(1–7) is able to counterbalance the effects of Ang II, resulting in a dynamic equilibrium between the levels of Ang-(1–7) and Ang II (van Twist et al. 2014).

Ang-(1–7) has several other beneficial and protector effects on cardiovascular diseases. For example, several animal studies have demonstrated that blockade of the ACE2/Ang-(1–7)/Mas axis results in cardiac injury, in particular increased inflammation, fibrosis and apoptotic responses (Meng et al. 2014; van Twist et al. 2014). Together with the fact that ACE2 activity in humans is associated with better left ventricular function and improved outcomes in cases of acute heart failure (Hao et al. 2013; van Twist et al. 2014), these findings indicate that ACE2/Ang-(1–7)/Mas stimulation could play a

beneficial role in cardiac remodeling and the treatment of heart failure (van Twist et al. 2014).

Recently, alamandine, a product of decarboxylation of the N-terminal Asp residue of Ang II to form angiotensin A, which is subsequently converted by ACE2 (Simões et al. 2016), was included in the *good axis* of the alternative RAAS. This new angiotensin peptide has similar properties to Ang-(1–7) and acts via the MrgD receptor (Villela et al. 2014). The only difference in the chemical structure of alamandine and Ang-(1–7) is the substitution of the N-terminal Asp residue by Ala (Simões et al. 2016). Interestingly, hypertensive animals treated with alamandine presented blood pressure reduction compared with normotensive control animals (Lautner et al. 2013). Despite these novel findings, the effects of this new RAAS component should be further explored.

RAAS and the adipose tissue

In addition to all the effects of RAAS classic or alternative pathways in the cardiovascular system, in the last three decades there has been a scientific focus to understand why this system is also present in white adipocytes. The expression of all mediators of the renin–angiotensin system in adipose tissue has been implicated in the modulation of adipocyte formation, glucose metabolism, triglyceride accumulation, lipolysis and the onset of the adverse metabolic consequences of obesity (Dünner et al. 2013). Moreover, in obese subjects, the systemic and the adipose higher levels of AngII activate these metabolic changes and worsen the status by a cross-talk between adipocyte-derived AngII and aldosterone, worsening the deleterious effects of the metabolic syndrome (Cassis et al. 2008; Engeli et al. 1999; Jaffe and Mendelsohn 2005; Lemarie et al. 2008). It has been reported that cell membrane-associated renin-receptors are present in human adipose tissue, specifically synthesized in the stromal portion of human adipose tissue in both isolated interadipocyte stromal cells and in stromal areas (Achard et al. 2007). The binding to these receptors evokes enhanced renin enzymatic activity that results in increased local and, consequently, systemic Ang II formation (Marcus et al. 2013; Nguyen 2011). Karlsson et al. (1998) demonstrated that angiotensinogen and ACE can be found in the adipose tissue from obese humans, raising the possibility that Ang II was made locally and could exert local effects on insulin resistance (Weir and Dzau 1999). It is well established that the adipocytes express angiotensin receptors (Putnam et al. 2012) and, recently, it was described that dysfunctional AT1R leads to altered adipogenesis (Weir and Dzau 1999).

To clarify the effects of Ang II through the AT1 receptor on adipose tissue, Zorad et al. (2006) treated Wistar Kyoto rats with an agonist for this receptor for 18 weeks. The animals treated with this agonist presented reduced weight gain and decreased fat tissue mass. This result seems to be due to the hypotrophy of epididymal and retroperitoneal adipose tissue and decreased adipocyte size without changing the number of adipocytes. Goossens et al. (2007) demonstrated that Ang II inhibits lipolysis through the AT1 receptor in human isolated adipocytes, supporting the concept that the local RAAS in adipose tissue participates directly in fat metabolism and may contribute to the metabolic disturbances.

Gembardt at al. (2005) showed that ACE2 is expressed in adipose tissue, as well as in other tissues and that ACE2/Ang(1–7)/Mas signaling regulates various functions of adipocytes in an intriguing counteractive manner (Than et al. 2013). This alternative pathway has opposite effects from the ACE-Ang II- AT1 signaling, as it stimulates the lipolysis and insulin-induced glucose uptake in adipocytes and inhibits oxidative stress (Oh et al. 2012; Than et al. 2013; Liu et al. 2012). In vivo, activation of endogenous ACE 2 reduces fat deposition (de Macedo et al. 2015). Furthermore, oral Ang-(1–7) increased ACE 2 expression and reduced fat deposition in high-fat diet-treated rats (Santos et al. 2013). Oh et al. (2012) verified that Ang-(1–7) increased glycerol release from primary adipocytes in a dose-dependent manner and that the lipolytic effect of Ang-(1–7) was attenuated by pretreatment with a Mas receptor blocker and with an inhibitor of PI3K or eNOS in Sprague–Dawley rats. However, the treatment with AT1 and AT2 blockers in vitro did not induce any change in the Ang-(1–7)-stimulated lipolysis.

The aldosterone effects in the electrolyte balance and blood pressure by regulating trans-epithelial sodium transport are via the mineralocorticoid receptor (MR) (Funder 2005). Caprio et al. (2007) proved that the MR is also expressed in non-epithelial cells, including adipocytes, confirming the importance of the local RAAS. Moreover, epidemiological studies have shown a clear association between aldosterone levels and the incidence of metabolic syndrome, now that the MR over-activation has been proven to cause hypertension, endothelial inflammation, insulin resistance and altered function of adipose tissue (Marzolla et al. 2012).

Conclusion

The coexistence of cardiometabolic syndrome and disturbances in RAAS has been stated in many studies published over the years. However, the clear relationship between them still requires further elucidation. The classic treatments for hypertension and diabetes have already been described and are very effective but a new approach to understanding the role of the adipose tissue ratio and the RAAS may increase the range of treatments, opening up possibilities for combined treatments. The existence of related studies has helped to fill the gaps created by the newly isolated findings, allowing a real

perspective about how this system contributes to the development of cardiometabolic syndrome.

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Compliance with ethical standards

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References

Achard V, Boullu-Ciocca S, Desbriere R, Nguyen G, Grino M (2007) Renin receptor expression in human adipose tissue. Am J Physiol Regul Integr Comp Physiol 292:R274–R282

Atlas SA (2007) The renin-angiotensin aldosterone system: pathophysiological role and pharmacologic inhibition. J Manag Care Pharm 13:9–20

Beard KM, Lu H, Ho K, Fantus IG (2006) Bradykinin augments insulin-stimulated glucose transport in rat adipocytes via endothelial nitric oxide synthase-mediated inhibition of Jun NH2-terminal kinase. Diabetes 55:2678–2687

Brown M (2006) Direct renin inhibition—a new way of targeting the renin system. J Renin-Angiotensin-Aldosterone Syst 7:5

Caprio M, Fève B, Claës A, Viengchareun S, Lombès M, Zennaro MC (2007) Pivotal role of the mineralocorticoid receptor in corticosteroid-induced adipogenesis. FASEB J 21:2185–2194

Carey RM, Siragy HM (2003) Newly recognized components of the renin-angiotensin system: potential roles in cardiovascular and renal regulation. Endocr Rev 24:261–271

Carey RM, Howell NL, Jin XH, Siragy HM (2001) Angiotensin type 2 receptor-mediated hypotension in angiotensin type-1 receptor-blocked rats. Hypertension 38:1272–1277

Cassis LA, Police SB, Yiannikouris F, Thatcher SE (2008) Local adipose tissue renin-angiotensin system. Curr Hypertens Rep 10:93–98

de Macedo SM, Guimarares TA, Andrade JM, Guimaraes AL, Batista de Paula AM, Ferreira AJ, Sousa Santos SH (2015) Angiotensin converting enzyme 2 activator (DIZE) modulates metabolic profiles in mice, decreasing lipogenesis. Protein Pept Lett 22:332–340

Dünner N, Quezada C, Berndt FA, Cánovas J, Rojas CV (2013) Angiotensin II signaling in human preadipose cells: participation of ERK1,2-dependent modulation of Akt. PLoS ONE 8:e75440

Durik M, Sevá Pessôa B, Roks AJ (2012) The renin-angiotensin system, bone marrow and progenitor cells. Clin Sci (Lond) 123:205–223

Engeli S, Sharma AM (2001) The renin-angiotensin system and natriuretic peptides in obesity-associated hypertension. J Mol Med (Berl) 79:21–29

Engeli S, Gorzelniak K, Kreutz R, Runkel N, Distler A, Sharma AM (1999) Co-expression of renin-angiotensin system genes in human adipose tissue. J Hypertens 17:555–560

Epstein BJ, Leonard PT, Shah NK (2012) The evolving landscape of RAAS inhibition: from ACE inhibitors to ARBs, to DRIs and beyond. Expert Rev Cardiovasc Ther 10:713–725

Ferrario CM (2006) Role of angiotensin II in cardiovascular disease therapeutic implications of more than a century of research. J Renin-Angiotensin-Aldosterone Syst 7:3–14

Frigolet ME, Torres N, Tovar AR (2013) The renin-angiotensin system in adipose tissue and its metabolic consequences during obesity. J Nutr Biochem 24:2003–2015

Funder JW (2002) New biology of aldosterone, and experimental studies on the selective aldosterone blocker eplerenone. Am Heart J 144:S8–S11

Funder JW (2005) Mineralocorticoid receptors: distribution and activation. Heart Fail Rev 10:15–22

Gembardt F, Sterner-Kock A, Imboden H, Spalteholz M, Reibitz F, Schultheiss HP, Siems WE, Walther T (2005) Organ-specific distribution of ACE2 mRNA and correlating peptidase activity in rodents. Peptides 26:1270–1277

Gironacci MM (2015) Angiotensin-(1–7): beyond its central effects on blood pressure. Ther Adv Cardiovasc Dis 9:209–216

Goossens GH, Blaak EE, Arner P, Saris WH, van Baak MA (2007) Angiotensin II: a hormone that affects lipid metabolism in adipose tissue. Int J Obes (Lond) 31:382–384

Hao PP, Chen YG, Liu YP, Zhang MX, Yang JM, Gao F, Zhang Y, Zhang C (2013) Association of plasma angiotensin-(1–7) level and left ventricular function in patients with type 2 diabetes mellitus. PLoS ONE 8:e62788

Jacome-Sosa M, Parks EJ, Bruno RS, Tasali E, Lewis GF, Schneeman BO, Rains TM (2016) Postprandial metabolism of macronutrients and cardiometabolic risk: recent developments, emerging concepts, and future directions. Adv Nutr 7:364–374

Jaffe IZ, Mendelsohn ME (2005) Angiotensin II and aldosterone regulate gene transcription via functional mineralocortocoid receptors in human coronary artery smooth muscle cells. Circ Res 96:643–650

Jing F, Mogi M, Horiuchi M (2013) Role of renin-angiotensin-aldosterone system in adipose tissue dysfunction. Mol Cell Endocrinol 378:23–28

Karlsson C, Lindell K, Ottosson M, Sjöström L, Carlsson B, Carlsson LM (1998) Human adipose tissue expresses angiotensinogen and enzymes required for its conversion to angiotensin II. J Clin Endocrinol Metab 83:3925–3929

Kintscher U, Law RE (2005) PPARgamma-mediated insulin sensitization: the importance of fat versus muscle. Am J Physiol Endocrinol Metab 288:E287–E291

Lautner RQ, Villela DC, Fraga-Silva RA, Silva N, Verano-Braga T, Costa-Fraga F, Jankowski J, Jankowski V, Sousa F, Alzamora A, Soares E, Barbosa C, Kjeldsen F, Oliveira A, Braga J, Savergnini S, Maia G, Peluso AB, Passos-Silva D, Ferreira A, Alves F, Martins A, Raizada M, Paula R, Motta-Santos D, Klempin F, Pimenta A, Alenina N, Sinisterra R, Bader M, Campagnole-Santos MJ, Santos RA (2013) Discovery and characterization of alamandine: a novel component of the renin-angiotensin system. Circ Res 112:1104–1111

Lemarie CA, Paradis P, Schiffrin EL (2008) New insights on signaling cascades induced by cross-talk between angiotensin II and aldosterone. J Mol Med (Berl) 86:673–678

Liu C, Lv XH, Li HX, Cao X, Zhang F, Wang L, Yu M, Yang JK (2012) Angiotensin-(1–7) suppresses oxidative stress and improves glucose uptake via Mas receptor in adipocytes. Acta Diabetol 49:291–299

Maleki M, Nematbakhsh M (2016) Gender difference in renal blood flow response to angiotensin II administration after ischemia/reperfusion in rats: the role of AT2 receptor. Adv Pharmacol Sci 2016:7294942

Marcus Y, Shefer G, Stern N (2013) Adipose tissue renin-angiotensin-aldosterone system (RAAS) and progression of insulin resistance. Mol Cell Endocrinol 378:1–14

Marzolla V, Armani A, Zennaro MC, Cinti F, Mammi C, Fabbri A, Rosano GM, Caprio M (2012) The role of the mineralocorticoid receptor in adipocyte biology and fat metabolism. Mol Cell Endocrinol 350:281–288

Matfin G (2007) Biomarkers in clinical trials and drug development: measurement of cardiometabolic risk. In: Division of Endocrinology and Diabetes DoM, New York University School of Medicine (ed), vol 7, British Journal of Diabetes & Vascular Disease, pp 101–106

McCarty MF (2003) ACE inhibition may decrease diabetes risk by boosting the impact of bradykinin on adipocytes. Med Hypotheses 60:779–783

Ménard J, Campbell DJ, Azizi M, Gonzales MF (1997) Synergistic effects of ACE inhibition and Ang II antagonism on blood pressure, cardiac weight, and renin in spontaneously hypertensive rats. Circulation 96:3072–3078

Meng W, Zhao W, Zhao T, Liu C, Chen Y, Liu H, Sun Y (2014) Autocrine and paracrine function of Angiotensin 1–7 in tissue repair during hypertension. Am J Hypertens 27:775–782

Nagel JM, Tietz AB, Göke B, Parhofer KG (2006) The effect of telmisartan on glucose and lipid metabolism in nondiabetic, insulin-resistant subjects. Metabolism 55:1149–1154

Nguyen G (2011) Renin and prorenin receptor in hypertension: what's new? Curr Hypertens Rep 13:79–85

Oh YB, Kim JH, Park BM, Park BH, Kim SH (2012) Captopril intake decreases body weight gain via angiotensin-(1–7). Peptides 37:79–85

Paulis L, Unger T (2010) Novel therapeutic targets for hypertension. Nat Rev Cardiol 7:431–441

Perk J, De Backer G, Gohlke H, Graham I, Reiner Z, Verschuren M, Albus C, Benlian P, Boysen G, Cifkova R, Deaton C, Ebrahim S, Fisher M, Germano G, Hobbs R, Hoes A, Karadeniz S, Mezzani A, Prescott E, Ryden L, Scherer M, Syvanne M, op Reimer WJ S, Vrints C, Wood D, Zamorano JL, Zannad F, European Association for Cardiovascular P, Rehabilitation, Guidelines ESCCfP (2012) European guidelines on cardiovascular disease prevention in clinical practice (version 2012). The Fifth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of nine societies and by invited experts). Eur Heart J 33:1635–1701

Putnam K, Batifoulier-Yiannikouris F, Bharadwaj KG, Lewis E, Karounos M, Daugherty A, Cassis LA (2012) Deficiency of angiotensin type 1a receptors in adipocytes reduces differentiation and promotes hypertrophy of adipocytes in lean mice. Endocrinology 153:4677–4686

Sampson AK, Hilliard LM, Moritz KM, Thomas MC, Tikellis C, Widdop RE, Denton KM (2012) The arterial depressor response to chronic low-dose angiotensin II infusion in female rats is estrogen dependent. Am J Physiol Regul Integr Comp Physiol 302:R159–R165

Santos SH, Andrade JM, Fernandes LR, Sinisterra RD, Sousa FB, Feltenberger JD, Alvarez-Leite JI, Santos RA (2013) Oral Angiotensin-(1–7) prevented obesity and hepatic inflammation by inhibition of resistin/TLR4/MAPK/NF-kappaB in rats fed with high-fat diet. Peptides 46:47–52

Seva Pessoa B, van der Lubbe N, Verdonk K, Roks AJ, Hoorn EJ, Danser AH (2013) Key developments in renin-angiotensin-aldosterone system inhibition. Nat Rev Nephrol 9:26–36

Simões E, Silva AC, Teixeira MM (2016) ACE inhibition, ACE2 and angiotensin-(1–7) axis in kidney and cardiac inflammation and fibrosis. Pharmacol Res 107:154–162

Siragy HM, Carey RM (1997) The subtype 2 (AT2) angiotensin receptor mediates renal production of nitric oxide in conscious rats. J Clin Invest 100:264–269

Takahashi H, Yoshika M, Komiyama Y, Nishimura M (2011) The central mechanism underlying hypertension: a review of the roles of sodium ions, epithelial sodium channels, the renin-angiotensin-aldosterone system, oxidative stress and endogenous digitalis in the brain. Hypertens Res 34:1147–1160

Than A, Leow MK, Chen P (2013) Control of adipogenesis by the autocrine interplays between angiotensin 1-7/Mas receptor and angiotensin II/AT1 receptor signaling pathways. J Biol Chem 288:15520–15531

Unger T, Paulis L, Sica DA (2011) Therapeutic perspectives in hypertension: novel means for renin-angiotensin-aldosterone system modulation and emerging device-based approaches. Eur Heart J 32:2739–2747

van Twist DJ, Kroon AA, de Leeuw PW (2014) Angiotensin-(1–7) as a strategy in the treatment of hypertension? Curr Opin Nephrol Hypertens 23:480–486

Verano-Braga T, Schwämmle V, Sylvester M, Passos-Silva DG, Peluso AA, Etelvino GM, Santos RA, Roepstorff P (2012) Time-resolved quantitative phosphoproteomics: new insights into Angiotensin-(1–7) signaling networks in human endothelial cells. J Proteome Res 11:3370–3381

Vermes E, Ducharme A, Bourassa MG, Lessard M, White M, Tardif JC, Dysfunction SOLV (2003) Enalapril reduces the incidence of diabetes in patients with chronic heart failure: insight from the studies of left ventricular dysfunction (SOLVD). Circulation 107:1291–1296

Villela DC, Passos-Silva DG, Santos RA (2014) Alamandine: a new member of the angiotensin family. Curr Opin Nephrol Hypertens 23:130–134

Wei Y, Zavilowitz B, Satlin LM, Wang WH (2007) Angiotensin II inhibits the ROMK-like small conductance K channel in renal cortical collecting duct during dietary potassium restriction. J Biol Chem 282:6455–6462

Weir MR, Dzau VJ (1999) The renin-angiotensin-aldosterone system: a specific target for hypertension management. Am J Hypertens 12: 205S–213S

Whaley-Connell A, Pavey BS, Chaudhary K, Saab G, Sowers JR (2007) Renin-angiotensin-aldosterone system intervention in the cardiometabolic syndrome and cardio-renal protection. Ther Adv Cardiovasc Dis 1:27–35

Yue P, Sun P, Lin DH, Pan C, Xing W, Wang W (2011) Angiotensin II diminishes the effect of SGK1 on the WNK4-mediated inhibition of ROMK1 channels. Kidney Int 79:423–431

Zorad S, Dou JT, Benicky J, Hutanu D, Tybitanclova K, Zhou J, Saavedra JM (2006) Long-term angiotensin II AT1 receptor inhibition produces adipose tissue hypotrophy accompanied by increased expression of adiponectin and PPARgamma. Eur J Pharmacol 552:112–122

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RESUMOS EM CONGRESSOS

CONGRESSO ABERTO AOS ESTUDANTES DE BIOLOGIA
24 a 28 Julho de 2017 Unicamp - Campinas

PESO CORPORAL, INGESTA HÍDRICA E ALIMENTAR: AVALIAÇÃO EM RATO ESPONTANEAMENTE HIPERTENSO E SEU CONTROLE, O WISTAR-KYOTO

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Palavras-Chave: *Ingesta; SHR; Wistar-Kyoto*

INTRODUÇÃO

Os ratos espontaneamente hipertensos (SHR) são animais que desenvolvem um quadro multifatorial semelhante à hipertensão essencial em humanos. O desenvolvimento da hipertensão em SHR é caracterizado pelo aumento na atividade simpatoadrenal que acontece entre o 4ª e 13ª semana de idade, que é seguida pelo aumento na pressão arterial. Os SHR apresentam, dentre uma série de diferenças, maior ativação no eixo hipotálamo-hipófise-tireoide (HPT), porém suas concentrações plasmáticas de catecolaminas não se distinguem de seu controle, o Wistar-Kyoto (WKY).

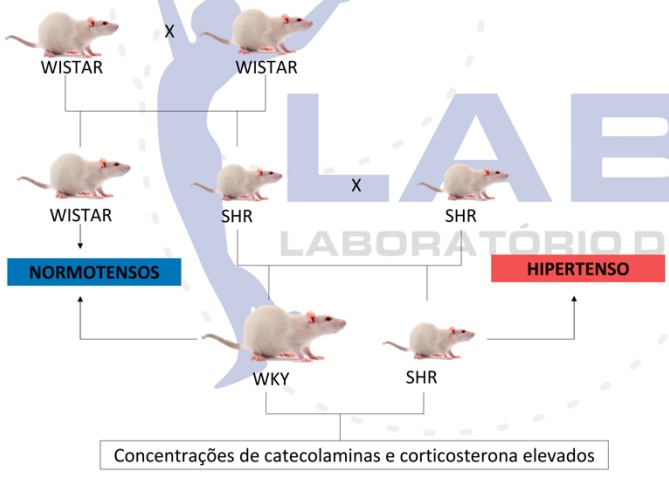

Figura 1: Esquema do desenho experimental do cruzamento das linhagens de ratos.

OBJETIVOS

Avaliar o peso corporal e os consumos hídrico e alimentar de ratos WKY e SHR.

MÉTODOS

Foi monitorado peso, ingesta hídrica e alimentar em WKY (n=6) e SHR (n=6) entre a 10ª e a 15ª semana (S) de vida (Comitê de Ética 4073-1). Fornecemos *ad libitum* água filtrada e ração padrão. Devido à alometria (superfície/volume) distinta entre as linhagens, os valores foram apresentados por grama (g) de animal, ie: alimento=g ração e hídrico água=mL, ambos por g peso. A normalidade foi testada por Kolmogorov-Smirnov. Foi aplicado Test t-Student para dados paramétricos e Mann-Whitney quando não paramétricos. Considerou-se o valor p<0,05.

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RESULTADOS

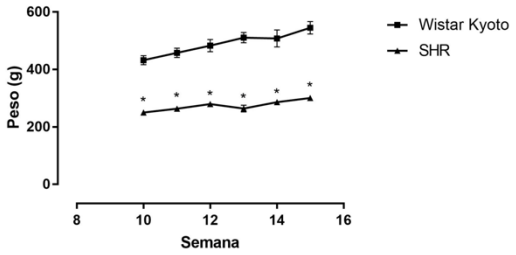

Figura 2: Peso WKY e SHR entre a 10ª e 15ª semana de vida. *p< 0,05 vs WKY.

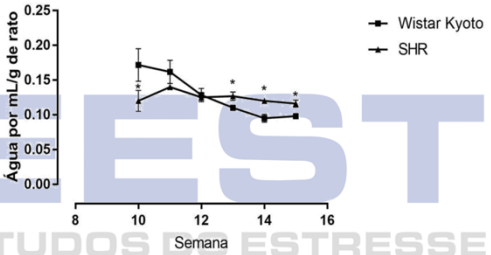

Figura 3: Ingesta hídrica WKY e SHR entre a 10ª e 15ª semana de vida. *p< 0,05 vs WKY.

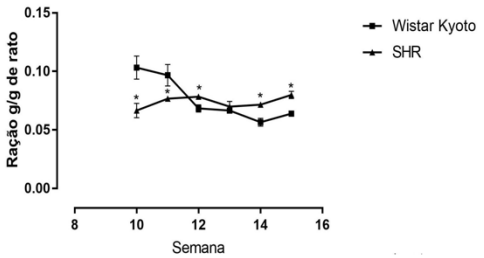

Figura 4: Ingesta alimentar WKY e SHR entre a 10ª e 15ª semana de vida. *p< 0,05 vs WKY.

CONCLUSÃO

O menor peso em relação ao WKY, associado a uma ingesta maior de água e alimentos, apontam um metabolismo energético acelerado em SHR. Estes resultados estão relacionados à maior ativação do eixo HPT que estes animais apresentam após a 10ª semana de vida.

APOIO

CAPES PRP unicamp FAEPEX FAPESP SAE serviço de apoio ao estudante

CONGRESSO ABERTO AOS ESTUDANTES DE BIOLOGIA
24 a 28 Julho de 2017 Unicamp - Campinas

PESO CORPORAL, INGESTA HÍDRICA E ALIMENTAR ENTRE RATOS NORMOTENSOS: WISTAR E WISTAR-KYOTO

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Laboratório de Estudos do Estresse - Labeest, Departamento de Biologia Estrutural e Funcional, Instituto de Biologia, Unicamp.
***Autor de correspondência:** doramgk@unicamp.br
Palavras-Chave: *Ingesta; Wistar-Kyoto; Wistar*

INTRODUÇÃO

Os ratos da linhagem Wistar (W) são utilizados como controle na maioria dos estudos. Os ratos Wistar-Kyoto (WKY) foram desenvolvidos como controle de uma linhagem de ratos espontaneamente hipertensos (Figura 1). O WKY apresenta maior atividade dos hormônios relacionados ao estresse, ou seja, ativação no eixo hipotálamo-pituária-adrenal (HPA). Apesar desta alteração, o WKY é normotenso, mais pesado e tem maior área e diâmetro em seus panículos adiposos. Não há na literatura estudos com períodos de observação prolongados comparando consumo hídrico e alimentar destas duas linhagens.

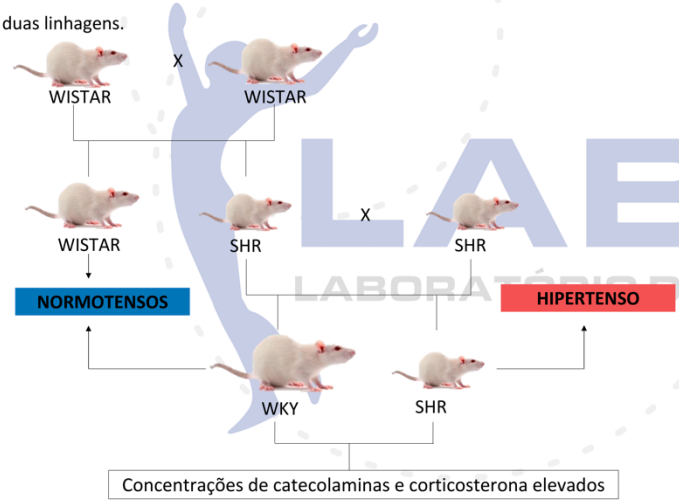

Figura 1. Esquema do desenho experimental do cruzamento das linhagens de ratos.

OBJETIVOS

Avaliar o peso corporal e os consumos hídrico e alimentar de W e WKY.

MÉTODOS

Foi monitorado peso, ingesta hídrica e alimentar em WKY (n=6) e W (n=6) entre a 10ª e a 15ª semana (S) de vida (Comitê de Ética 4073-1). Fornecemos *ad libitum* água filtrada e ração padrão. Devido à alometria (superfície/volume) distinta entre as linhagens, os valores foram apresentados por grama (g) de animal, ie: alimento=g ração e hídrico água=mL, ambos por g de peso. A normalidade foi testada por Kolmogorov-Smirnov. Foi aplicado Test t-Student para dados paramétricos e Mann-Whitney quando não paramétricos. Considerou-se o valor p<0,05.

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RESULTADOS

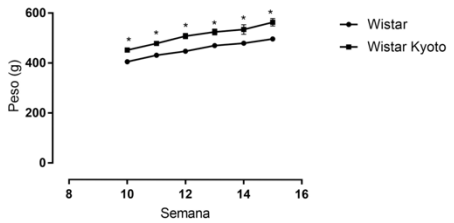

Figura 2. Peso Wistar e WKY entre a 10ª e 15ª semana de vida de vida. *p< 0,05 vs W.

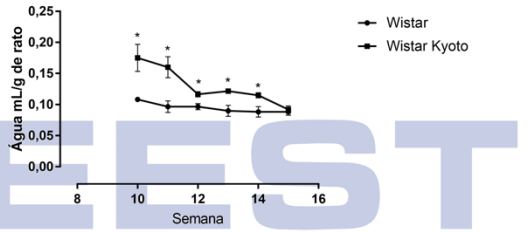

Figura 3. Ingesta hídrica Wistar e WKY entre a 10ª e 15ª semana de vida. *p< 0,05 vs W.

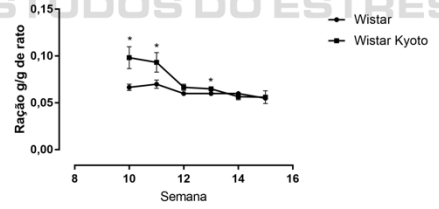

Figura 4. Ingesta alimentar Wistar e WKY entre a 10ª e 15ª semana de vida. *p< 0,05 vs W.

CONCLUSÃO

O maior ganho de peso do WKY associado a uma ingesta inicial maior de ração apontam um metabolismo energético semelhante entre as duas linhagens. Como WKY apresenta maior consumo hídrico, sugerimos que o desajuste no eixo HPA leva a maior produção de aldosterona e consequentemente maior retenção de sal, aumentando a sede e a produção do hormônio antidiurético. São necessárias investigações hormonais para validar esta hipótese.

APOIO

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Urinalysis of Wistar, Wistar Kyoto and Spontaneous Hypertensive Rats

Carolina Silva, Filipy Borghi, Camila L. Morais, Priscila C. da Silva, Dora M. Grassi-Kassisse*

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Abstract

Urinalysis reveals information about metabolic, hepatic, and renal function as well as indications of the dysfunction etiology. It is a simple, practical and quick method to perform analysis of the biochemical urine constituents by markers in reagent strips. In this study, we evaluated the urine of Wistar, Wistar Kyoto (WKY) and spontaneously hypertensive rats (SHR). Urinalysis of WKY and SHR rats indicated ketosis and increased protein presence than Wistar. The WKY and SHR urine was more diluted than Wistar. Only the WKY presented hemolysis. The results indicate absence of hepatic dysfunction and infections in the urine of the different strains of rats. Ketonuria corroborates with the fasting rat's state, hematuria is an indicative of the urinary system dysfunction and proteinuria specifically refers to renal dysfunction, these are related to high WKY weight and SHR hypertension. Dilution of urine may be related to hydroelectrolytic imbalance due to changes in the HPA axis presented by WKY and SHR

Keywords: Urine markers; urinalysis; rats.

Introduction

Urine is an easily obtainable fluid. The urinalysis reveals information about the general function of the organism related to the metabolic, hepatic and renal state, providing indications on the dysfunction etiology (1). This analysis is a fast, practical and low-cost method. Frequently, reagent strips are used for the analysis of the biochemical constituents of urine which are a simple and rapid way of performing ten or more analyzes of important biochemical markers, such as urobilinogen, proteins and ketones (1). SHR rats (spontaneously hypertensive), developed by Okamoto & Aoki (2), present a multifactorial condition extremely similar to the essential hypertension in human (3) and have the Wistar Kyoto (WKY) as control that is heavier than the SHR and Wistar rats. WKY, as well as SHR, exhibit increased activation in the hypothalamic-pituitary-adrenal axis (HPA) (4), however, they are normotensive, such as the Wistar. We aimed to evaluate the metabolic, hepatic and renal function of 15 weeks old Wistar, WKY and SHR rats from urine samples.

Results and Discussion

Urinalysis (Figure 1) of WKY and SHR rats indicated ketosis and increased protein presence than Wistar. The urine of WKY and SHR was more diluted than Wistar. Only WKY showed hemolysis (Table 1).

Table 1- Urinalysis in 15-week-old Wistar, WKY and SHR rats.

Markers	Wistar	WKY	SHR
Protein	22.5 (± 2.835)	30 (+) 0.3	30 (+) 0.3
Ketones	NEG	5 (0.5)	5 (0.5)
Blood	NEG	Hemolysis traits	NEG
Leukocytes	NEG	NEG	NEG
pH	7	7	7
Nitrites	NEG	NEG	NEG
Urobilinogen	NORMAL	NORMAL	NORMAL
Density	1.020	1.010	1.010
Bilirubin	NEG	NEG	NEG
Glucose	NEG	NEG	NEG

Figure 1- Image of urine markers and a result example.

Conclusions

Prolonged fasting promotes the lipid metabolism, which have as byproduct ketone bodies. Ketonuria corroborates to the fasted rats' status. Hematuria is a sign of urinary system dysfunction and proteinuria specifically refers to renal dysfunction. These results are related to the high WKY weight (5) and the hypertension presented by SHR (6). The exams indicated absence of hepatic dysfunction and infections in the urine of the different strains. The decrease in specific density may be related to hydroelectrolytic imbalance due to changes in the HPA axis presented by WKY and SHR.

Acknowledgment

FAPESP, FAEPEX, CAPES, SAE, PIBIC – CNPQ

1. Lima OA, B. SJ, B. GJ, J. G, M CJ. Métodos de laboratório aplicados à clínica: Técnica e interpretação: Guanabara Koogan; 2001.
2. Okamoto K, Aoki K. Development of a strain of spontaneously hypertensive rats. Jpn Circ J. 1963;27:282-93.
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The relationship of angiotensin II in energy metabolism of adipocytes: production of lactate and glycerol

Camila L. Morais, Filipy Borghi, Carolina Silva, Priscila C. da Silva, Dora M. Grassi-Kassisse*

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Abstract

Angiotensin II (AII) produced by Renin-Angiotensin-Aldosterone System (RAAS) through the classical pathway have been associated with the regulation of adipocyte glycolytic and lipolytic metabolism. This pathway can be potentiated by the increase of catecholamines. The Wistar-Kyoto (WKY) and Spontaneously Hypertensive Rats (SHR) strains exhibit high catecholamines pattern and have different adipocyte profiles. This work aimed to evaluate the influence of AII in the production of glycerol and lactate from these different strains and our results demonstrated that AII does not affect the glycolytic metabolism. However, AII seems to have anti-lipolytic effect in WKY isolated adipocytes.

Keywords: Angiotensin II, glycerol, lactate.

Introduction

Lipolytic and glycolytic activity are regulated by catecholamines, which act on the availability of stored energy, which are also involved with the Renin-Angiotensin-Aldosterone System (RAAS) activation, responsible for angiotensin II (AII) production in the classic pathway (1,2). Therefore, the AII may be related to lipolysis and glycolysis modulation in adipose tissue, where the AT1 and AT2 receptors blockade affect the adipocyte metabolism (3). The Spontaneously Hypertensive Rats (SHR), as its control Wistar-Kyoto (WKY), exhibit high catecholamines pattern and different weights and adipocytes profiles, which WKY is heavier, with higher area and diameter of their adipocytes (4). This work aims to evaluate the influence of AII in the production of glycerol and lactate from these different strains.

Results and Discussion

Ethical Committee approved the protocol under number 4073-1. Male 15-week-old Wistar (WIS), WKY and SHR rats (n=5-12) were used for the experiments. The normality was confirmed by Shapiro-Wilk test and then we performed Student's t-test for parametric and Mann-Whitney for nonparametric data. Our results were standardized by area due to the fact that the size influences the adipocyte metabolism. There is no difference between strains in the basal glycerol production (Figure 1A). AII did not increase significantly the glycerol production above basal levels (Figure 1B). However, SHR exhibited higher glycerol levels in presence of AII (Figure 1B) probably due to a decrease in glycerol production in WKY isolated adipocytes under the same stimulus (10^{-7}M) (Figure 1B).

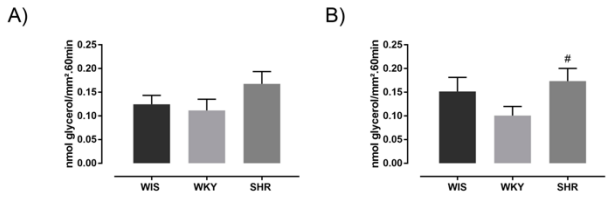

Figure 1. A) Basal production of glycerol from WIS, WKY and SHR; B) Basal production of glycerol incubated with AII (10^{-7}M) from WIS, WKY and SHR. p<0.05. n= 11-12

SHR exhibited higher basal lactate than the other strains (Figure 2A) and AII (10^{-7}M) does not affect the glycolytic activity in basal levels (Figure 2B)

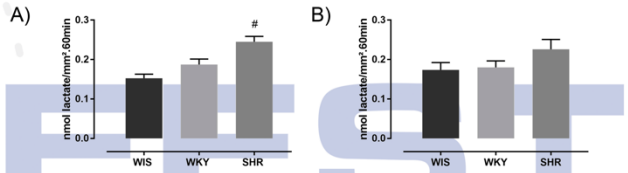

Figure 2. A) Basal production of lactate from WIS, WKY and SHR; B) Basal production of lactate incubated with AII (10^{-7}M) from WIS, WKY and SHR. p<0.05. n= 5-6

Conclusions

The lipolytic activity does not exhibit any difference in the basal production. However, AII seems to have anti-lipolytic in WKY isolated adipocytes. The glycolytic metabolism of the adipocyte is not affected by AII. Nevertheless, SHR presents high basal lactate in relation to the other strains, which may be due to increased LDH activity and higher α2 renal receptors density, which is associated with increased lactate efflux (5-7).

Acknowledgment

FAPESP, FAEPEX, CAPES, SAE, PIBIC – CNPQ

1. Borghi F, Sevá-Pessôa B, Grassi-Kassisse DM. The adipose tissue and the involvement of the renin-angiotensin-aldosterone system in cardiometabolic syndrome. Cell Tissue Res. 2016;366(3):543-8.
2. Takahashi H, Yoshika M, Komiyama Y, Nishimura M. The central mechanism underlying hypertension: a review of the roles of sodium ions, epithelial sodium channels, the renin-angiotensin-aldosterone system, oxidative stress and endogenous digitalis in the brain. Hypertens Res. 2011;34(11):114760.
3. Caminhotto ReO, Sertié RA, Andreotti S, Campaãa AB, Lima FB. Renin-angiotensin system blockers regulate the metabolism of isolated fat cells in vitro. Braz J Med Biol Res. 2016;49(8).
4. Costa GT, Ishizu LY, Conceição-Vertamatti AG, Grassi-Kassisse DM. Morphometry of isolated adipocytes from rats of two models of hypertension. XXIII Congresso de Iniciação Científica da Unicamp.2015.
5. Zhou RY, Wang JJ, You Y, Sun JC, Song YC, Yuan HX, et al. [Effect of baicalin on ATPase and LDH and its regulatory effect on the AC/cAMP/PKA signaling pathway in rats with attention deficit hyperactivity disorder]. Zhongguo Dang Dai Er Ke Za Zhi. 2017;19(5):576-82.
6. Kopp UC, Cicha MZ, Smith LA. Impaired interaction between efferent and afferent renal nerve activity in SHR involves increased activation of alpha2-adrenoceptors. Hypertension. 2011;57(3):640-7.
7. Lockette W, Kirkland K, Farrow S. Alpha 2-adrenergic agonists increase cellular lactate efflux. Hypertension. 1996;27(5):1104-7.

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The relationship of angiotensin II in energy metabolism of adipocytes: production of lactate and glycerol

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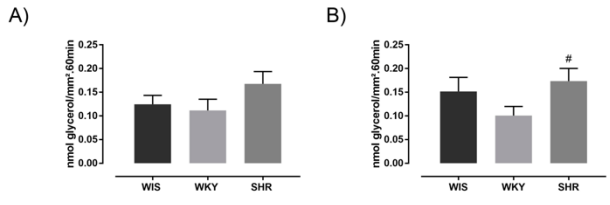

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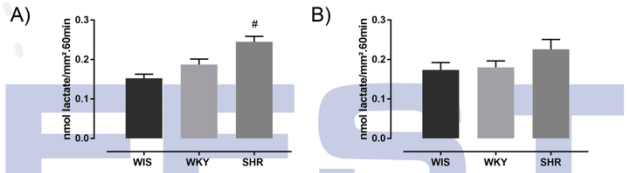

Figure 2. A) Basal production of lactate from WIS, WKY and SHR; B) Basal production of lactate incubated with AII (10^{-7}M) from WIS, WKY and SHR. p<0.05. n= 5-6

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Signaling downstream cascade crosstalk induced by noradrenaline on angiotensin II metabolic activity in adipocytes.

Camila L. Morais*, Filipy Borghi, Carolina Silva, Priscila C. da Silva, Dora Maria Grassi-Kassisse.

Abstract

The role of angiotensin II (AII) on lipolytic metabolism is still controversial in the literature. Diversely, noradrenaline (NOR) is an important stimulator of lipolysis and glycolysis in adipocytes. Spontaneously hypertensive rats (SHR) have lower body weight and smaller adipocytes when compared to its control, Wistar-Kyoto, with higher basal lactate production by area and volume. We aimed in this work to evaluate the influence in downstream cascate of AII and NOR, co-incubated or not, in the production of glycerol and lactate in three different strains. The combination of NOR and AII potentiated the lipolytic activity by decreasing the glucose metabolism to lactate. This effect may be associated with the NOR activity on adrenergic receptors or by the AII activity blockade at AT1 receptors, highlighting the antilipolytic activity of this receptor and the crosstalk between downstream cascade induced by these agonists.

Key words: *Angiotensin II; noradrenaline; glycerol; lactate.*

Introduction

The role of angiotensin II (AII) as a stimulator or inhibitor on lipolytic activity remains unclear in the literature[1,2]. Noradrenaline (NOR) has an important role in the energy metabolism of adipose tissue and exhibited a positive stimulus for lipolytic and glycolytic activity, increasing lactate and glycerol production[3, 4]. Spontaneously hypertensive rats (SHR) have lower body weight and smaller adipocytes when compared to its control, Wistar-Kyoto, with higher basal lactate production by area and volume[5]. We aimed in this work to evaluate the influence of AII and NOR, co-incubated or not, in the production of glycerol and lactate in different strains.

Results and Discussion

Ethical Committee approved the protocol under number 4073-1. Male 15-week-old Wistar (WIS), WKY and SHR rats (n=5-12) were used for the experiments. The normality was confirmed by Shapiro-Wilk test and then we performed Student's t-test for parametric and Mann-Whitney for nonparametric data. The results are expressed in nmol/mm^2.60min. Glycerol production increased in all strains under NOR stimulation at [10^{-7}] (WIS - 10^{-7}: 0.1488, 10^{-6}: 0.5159; WKY - 10^{-7}: 0.1664, 10^{-6}: 0.3513; SHR - 10^{-7}: 0.2145, 10^{-6}: 0.6868) and for the combined action of NOR+AII (WIS - 10^{-7}: 0.2668, 10^{-6}: 0.4152; WKY - 10^{-7}: 0.154, 10^{-6}: 0.403; SHR - 10^{-7}: 0.2403, 10^{-6}: 0.5095). Under influence of AII, no changes were observed. Lactate production did not change under any conditions in WIS, but WKY exhibited an increase in presence of NOR at [10^{-7}] (10^{-7}: 0.193; 10^{-6}: 0.2368) and a decrease in NOR+AII co-incubation (10^{-9}: 0.2849; 10^{-7}: 0.1835). There were no changes observed under influence of AII. SHR only showed a decrease in lactate production under co-incubation of NOR+AII (10^{-6}: 0.2279, 10^{-4}: 0.1696).

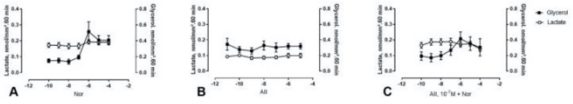

Figure 1 – Glycerol and lactate production in Wistar under influence of: A) NOR; B) AII; C) NOR+AII.

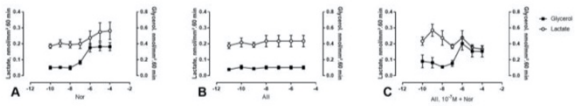

Figure 2 – Glycerol and lactate production in Wistar-Kyoto under influence of: A) NOR; B) AII; C) NOR+AII.

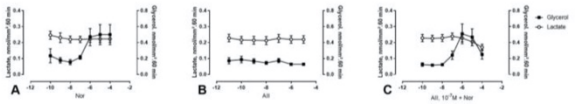

Figure 3 – Glycerol and lactate production in SHR under influence of: A) NOR; B) AII; C) NOR+AII.

Conclusions

We confirmed that NOR exhibited a positive stimulus in the lactate and glycerol production. The isolated action of AII can not be observed, but its combination with NOR potentiated the lipolytic activity by decreasing the glucose metabolism to lactate. This effect may be associated with the NOR activity on adrenergic receptors[4] or by the AII activity blockade at AT1 receptors, highlighting the antilipolytic activity of this receptor[7] and the crosstalk between downstream cascade induced by these agonists.

Acknowledgement

FAPESP, FAEPEX, CAPES, SAE, PIBIC – CNPQ

1. Boschmann M, Ringel J, Klaus S, Sharma AM. Metabolic and hemodynamic response of adipose tissue to angiotensin II. Obes Res. 2001;9(8):486-91.
2. Boschmann M, Jordan J, Adams F, Christensen NJ, Tank J, Franke G, et al. Tissue-specific response to interstitial angiotensin II in humans. Hypertension. 2003;41(1):37-41.
3. Faintrenie G, Géloën A. Alpha-1 adrenergic regulation of lactate production by white adipocytes. J Pharmacol Exp Ther. 1996;277(1):235-8.
4. Bartness TJ, Liu Y, Shrestha YB, Ryu V. Neural innervation of white adipose tissue and the control of lipolysis. Front Neuroendocrinol. 2014;35(4):473-93.
5. Morais CL, Borghi F, Silva C, Da Silva PC, Grassi-Kassisse DM. The relationship of angiotensin II in energy metabolism of adipocytes: production of lactate and glycerol. XXVI Congresso PIBIC2018 [
6. Borghi F, Sevá-Pessôa B, Grassi-Kassisse DM. The adipose tissue and the involvement of the renin-angiotensin-aldosterone system in cardiometabolic syndrome. Cell Tissue Res. 2016;366(3):543-8.