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CÂMPUS DE JABOTICABAL

PARTICIPAÇÃO DA IRISINA NO CONTROLE CARDIORRESPIRATÓRIO E METABÓLICO DE RATOS ADULTOS

Mariana Bernardes Ribeiro

Bióloga

2022

UNIVERSIDADE ESTADUAL PAULISTA - UNESP
CÂMPUS DE JABOTICABAL

PARTICIPAÇÃO DA IRISINA NO CONTROLE CARDIORRESPIRATÓRIO E METABÓLICO DE RATOS ADULTOS

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Orientadora: Profa. Dra. Luciane Helena Gargaglioni Batalhão

Dissertação apresentada à Faculdade de Ciências Agrárias e Veterinárias – UNESP, Câmpus de Jaboticabal, como parte das exigências para a obtenção do título de Mestre em Zootecnia

Julho de 2022

R484p

Ribeiro, Mariana Bernardes

Participação da irisina no controle cardiorrespiratório e metabólico de ratos adultos / Mariana Bernardes Ribeiro. -- Jaboticabal, 2022

72 f. : il., tabs.

Dissertação (mestrado) - Universidade Estadual Paulista (Unesp), Faculdade de Ciências Agrárias e Veterinárias, Jaboticabal

Orientadora: Luciane Helena Gargaglioni Batalhão

1. Fisiologia. 2. Ventilação. 3. Quimiorreceptores. 4. Metabolismo. I. Título.

Sistema de geração automática de fichas catalográficas da Unesp. Biblioteca da Faculdade de Ciências Agrárias e Veterinárias, Jaboticabal. Dados fornecidos pelo autor(a).

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TÍTULO DA DISSERTAÇÃO: PARTICIPAÇÃO DA IRISINA NO CONTROLE CARDIORRESPIRATÓRIO E METABÓLICO DE RATOS ADULTOS

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DADOS CURRICULARES DO AUTOR

Mariana Bernardes Ribeiro – nascida em 14 de julho de 1997 na cidade de Ituiutaba – MG, filha de Andrea Luciana Bernardes Marquez e de Renato Severino Ribeiro, ingressou no curso de Ciências Biológicas da Universidade Federal de Uberlândia -UFU, Campus Pontal em março de 2015. Durante o período de graduação, a aluna realizou duas iniciações científicas sendo uma delas financiada pela CNPq. O estágio curricular foi realizado no Laboratório de Anatomia e Fisiologia Humana do campus e no Laboratório de Fisiologia da Universidade Estadual Paulista "Júlio de Mesquita Filho" Campus Jaboticabal. Colou grau em agosto de 2019 e recebeu o título de Bacharel em Ciências Biológicas. Em março de 2020 iniciou o curso de Mestrado pelo Programa de Pós-graduação em Zootecnia no Departamento de Morfologia e Fisiologia Animal na Universidade Estadual Paulista – UNESP Campus Jaboticabal, onde foi bolsista CAPES e FAPESP, submetendo-se ao Exame Geral de Qualificação em 28 de setembro de 2020.

Dedico aos meus pais Andrea e Renato,
pilares da minha formação como ser humano.

AGRADECIMENTOS

Aos meus pais, Andrea e Renato, e a minha irmã Nathália, que sempre estiveram ao meu lado me apoiando em todas as etapas da minha vida, torcendo e acreditando na minha capacidade. Muito obrigada por todo carinho e compreensão pela minha ausência. Amo vocês!

Ao meu companheiro de vida, o meu melhor amigo, meu namorado Gilberto, por sempre se fazer presente em todos os momentos apesar da distância. Muito obrigada pela sua compreensão e incentivo. O seu amor e o seu apoio foram essenciais para a finalização deste trabalho.

À minha orientadora Luciane, por ter depositado em mim sua confiança, por me atender e me acolher com todo amor e paciência em todos os momentos que precisei. Agradeço imensamente por todos os ensinamentos e conselhos prestados. Minha eterna admiração e carinho por você, muito obrigada por tudo!

Ao meu co-orientador Luis Gustavo, por ter estado ao meu lado em todas as etapas de execução deste trabalho. Agradeço imensamente por todos os ensinamentos, pelas inúmeras cirurgias e análises feitas juntas, por ser paciente e me auxiliar nos momentos difíceis. Muito obrigada por todo tempo destinado a me ajudar, você foi fundamental!

Aos meus amigos e companheiros do laboratório: Sofia, Jessica, Thais, Livia, Geni, Aline, Elisa, Bia Felix, Bia Dominiquini, Ane, Carol, Gleyce, Gabriel, Leticia... muito obrigada por toda ajuda, pela companhia diária, pelos momentos de alegria e risadas e pelos momentos difíceis que vivemos e aprendemos juntos, ter vocês ao meu lado fez com o que o trabalho se tornasse muito mais agradável. Agradeço especialmente a Aline e a Elisa que estiveram ao meu lado no início dessa jornada, me auxiliando dentro e fora do laboratório, eu aprendi muito com vocês e serei eternamente grata. À Sofia, por toda companhia, amizade, ajuda, por todos os finais de semana no lab, por todas as conversas... Muito obrigada por me oferecer um ombro amigo sempre que precisei. A Jessica e a Thais por formar comigo o trio mais diverso que poderia existir, obrigada por todos os momentos juntos.

À profa. Kênia por todo auxílio, dicas e ensinamentos para o desenvolvimento deste trabalho.

Ao técnico Euclides pelo cuidado com os animais e a Ângela por todo auxílio na rotina do laboratório.

Aos animais que deram a vida para a realização deste estudo, o meu profundo respeito e gratidão.

O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Código de Financiamento 001 (processo nº88887.498167/2020-00).

Agradeço também a Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) pelo apoio financeiro e bolsa concedida (processo n° 2020/02918-9) que possibilitou a conclusão desse projeto com sucesso.

À Faculdade de Ciências Agrárias e Veterinárias – UNESP e o Programa de Pós-Graduação em Zootecnia pelo ensino e estrutura necessários para a execução do meu trabalho.

A todos que contribuíram de alguma forma para a realização deste trabalho, o meu muito obrigada!

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Câmpus de Jaboticabal

CEUA – COMISSÃO DE ÉTICA NO USO DE ANIMAIS

CERTIFICADO

Certificamos que o projeto de pesquisa intitulado **"Participação da irisina no controle cardiorrespiratório e metabólico de ratos adultos"**, protocolo nº 3337/20, sob a responsabilidade da Profª Drª Luciane Helena Gargaglione Batalhão, que envolve a produção, manutenção e/ou utilização de animais pertencentes ao Filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica (ou ensino) - encontra-se de acordo com os preceitos da lei nº 11.794, de 08 de outubro de 2008, no decreto 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA), e foi aprovado pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA), da FACULDADE DE CIÊNCIAS AGRÁRIAS E VETERINÁRIAS, UNESP - CÂMPUS DE JABOTICABAL-SP, em reunião extraordinária de 22 de maio de 2020.

Vigência do Projeto	02/06/2020 a 31/03/2022
Espécie / Linhagem	*Rattus norvegicus* – WISTAR
Nº de animais	120
Peso / Idade	250-350g
Sexo	Machos
Origem	Biotério Central do Câmpus da Unesp de Botucatu

Jaboticabal, 22 de maio de 2020.

Profª Drª Fabiana Pilarski
Coordenadora – CEUA

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PARTICIPAÇÃO DA IRISINA NO CONTROLE CARDIORRESPIRATÓRIO E METABÓLICO DE RATOS ADULTOS

Resumo: - A irisina é um hormônio constituído por 112 aminoácidos recém-identificado, derivado de uma proteína transmembrana codificada pelo gene fibronectina tipo III contendo 5 genes (FNDC5), que está altamente relacionado à atividade metabólica do músculo esquelético e do tecido adiposo, possuindo uma similaridade de 100% entre ratos e humanos. Sabe-se que a concentração da irisina aumenta durante o exercício físico trazendo efeitos benéficos para vários tecidos, inclusive para o sistema nervoso, promovendo neuroplasticidade, uma vez que a irisina parece estar associada a proliferação e diferenciação neural. Recentemente também foi demonstrado que esse hormônio modula as funções cardiovasculares podendo atuar como um importante mensageiro entre os órgãos para integrar o gasto de energia com a atividade cardiovascular. Entretanto, pouco se sabe sobre a participação da irisina no controle cardiorrespiratório e metabólico. Desta forma, o objetivo do presente estudo foi avaliar o efeito da injeção intracerebroventricular (icv) de veículo ou irisina (IR, 0,425 e 1,66 µg/µL) no quarto ventrículo (4V) de ratos adultos sobre ventilação ($\dot{V}_E$), consumo de oxigênio ($\dot{V}O_2$), equivalente respiratório ($\dot{V}_E / \dot{V}O_2$), temperatura corporal (T_C), pressão arterial média (PAM) e frequência cardíaca (FC) durante o estado de vigília e sono frente a exposição ao ar ambiente, hipercapnia (7% CO_2) e hipóxia (10% O_2). O tratamento com irisina centralmente com a menor concentração (0,425 µg/µL), promoveu uma redução na $\dot{V}_E$ em condições de ar ambiente durante a vigília, culminando em uma hipoventilação (menor $\dot{V}_E / \dot{V}O_2$). Durante a exposição as condições de ar ambiente, os animais tratados com a maior concentração de irisina (1,66 µg/µL), também apresentaram uma redução da $\dot{V}_E$ e da f_R. Os animais tratados com a maior concentração de irisina quando expostos a altos níveis de CO_2 apresentaram um aumento na $\dot{V}_E$, em decorrência de um maior V_T e uma redução no $\dot{V}O_2$, o que acarretou em uma maior hiperventilação (maior $\dot{V}_E / \dot{V}O_2$) durante a vigília. As duas concentrações de irisina atenuaram a hipotermia induzida pela hipóxia. Em relação aos parâmetros cardiovasculares, a injeção central de irisina na menor concentração causou um aumento significativo na FC em todas as condições durante o sono e a vigília, sem alterar a PAM. Enquanto o tratamento com a maior concentração, promoveu um aumento da FC nos animais quando expostos a hipóxia durante os estados de sono e vigília e durante a hipercapnia em estado de sono. Nossos dados demonstram que a irisina exerce um efeito inibitório no controle da respiração em condição de ar ambiente e potencializa a resposta hiperventilatória sob hipercapnia, promovendo um aumento da $\dot{V}_E$ e inibindo o $\dot{V}O_2$ durante a vigília. Além disso, essa miocina exerce uma modulação excitatória na FC e atenua a queda da temperatura corporal em condição de baixo O_2.

Palavras-chave: irisina, metabolismo, miocinas, ventilação

PARTICIPATION OF IRISIN IN CARDIORRESPIRATORY AND METABOLIC CONTROL OF ADULT RATS

Abstract: Irisin is a newly identified hormone consisting of 112 amino acids, derived from a transmembrane protein encoded by the type III fibronectin gene containing 5 (FNDC5), which is highly related to the metabolic activity of skeletal muscle and adipose tissue, having a similarity of 100 % between rats and humans. It is known that the concentration of irisin increases during physical exercise, bringing beneficial effects to various tissues, including the central nervous system, promoting neuroplasticity, since irisin seems to be associated with neural proliferation and differentiation. It has also recently been shown that this hormone modulates cardiovascular functions, possibly acting as an important messenger between organs to integrate energy expenditure with cardiovascular activity. Therefore, the present study evaluated the effect of intracerebroventricular (icv) injection of vehicle or irisin (IR, 0.425 and 1.66 µg/µL) in the fourth ventricle (4V) of adult male rats in ventilation ($\dot{V}_E$), oxygen consumption ($\dot{V}O_2$), ventilatory equivalent ($\dot{V}_E$ / $\dot{V}O_2$), body temperature (T_B), mean arterial pressure (MAP) and heart rate (HR) in wakefulness and sleep state during room air, hypercapnia (7% CO_2) and hypoxia (10% O_2). Treatment with irisin centrally with the lowest concentration (0.425 µg/µL), promoted a reduction in $\dot{V}_E$ in room air conditions during wakefulness, culminating in a hypoventilation (lower $\dot{V}_E$ / $\dot{V}O_2$). During exposure to room air conditions, animals treated with the highest concentration of irisin (1.66 µg/µL) also showed a reduction in $\dot{V}_E$ and f_R. The animals treated with the highest concentration of irisin when exposed to high levels of CO_2 showed an increase in $\dot{V}_E$, due to a higher V_T and a reduction in $\dot{V}O_2$, which resulted in hyperventilation (higher $\dot{V}_E$ / $\dot{V}O_2$) during wakefulness. The both concentrations of irisin attenuated hypoxia-induced regulated hypothermia. Regarding cardiovascular parameters, central irisin injection with the lower concentration caused a significant increase in HR under all conditions during sleep and wakefulness, without altering MAP. The treatment with the highest concentration promoted an increase in HR in the animals when exposed to hypoxia during sleep and wakefulness states and during hypercapnia in sleep state. Our data demonstrate that irisin exerts an inhibitory effect on breathing control and potentiates the hyperventilatory response under hypercapnia, promoting an increase in $\dot{V}_E$ and inhibiting $\dot{V}O_2$ during wakefulness. In addition, this myokine is excitatory to HR and attenuates the drop in body temperature in low O_2 conditions.

Keywords: Irisin, myokines, metabolism, ventilation

CAPÍTULO 1 – CONSIDERAÇÕES GERAIS

Introdução

A principal função da respiração é manter relativamente constante as pressões parciais arteriais de oxigênio (PaO_2) e dióxido de carbono ($PaCO_2$), bem como os valores de pH. Esse processo é altamente integrado e envolve uma complexa rede de interação entre encéfalo, medula espinhal, nervos cranianos e espinhais, musculaturas específicas e pulmões, além de neurotransmissores, neuromoduladores e receptores (Del Negro et al., 2018).

Por mais de um século, tem havido intenso interesse entre fisiologistas no mecanismo que regula a respiração durante o exercício. O fato muito reconhecido e incontestável é que em humanos saudáveis e na maioria dos outros mamíferos, a hiperpnéia do exercício não está associada ao aumento da $PaCO_2$ ou à uma diminuição na PaO_2 (Forster et al., 2012). Durante o exercício físico em indivíduos saudáveis, a ventilação alveolar e a difusão alvéolo-capilar se elevam proporcionalmente ao aumento da taxa metabólica para evitar alterações da $PaCO_2$ e PaO_2. Desta forma, ao menos em humanos, durante condições de exercício físico leve ou moderado, as pressões parciais dos gases sanguíneos se alteram minimamente, apesar do aumento da taxa metabólica em decorrência do esforço físico (Dempsey e Rankin, 1967; Forster et al., 1986). Até o presente momento, não há um mecanismo conhecido capaz de detectar diretamente a taxa de troca gasosa nos músculos ou nos pulmões (Forster et al., 2012). Assim, ainda não é totalmente conhecido como os neurônios respiratórios ajustam sua atividade frente ao exercício físico às variáveis que atualmente não podem ser diretamente monitoradas. Algumas hipóteses foram testadas e dados foram obtidos, porém para cada hipótese existem dados contraditórios ou razões para se questionar a validade de cada hipótese (Forster et al., 2012).

Nas últimas décadas, diversas pesquisas visaram avaliar a relação entre a contração muscular gerada durante o exercício físico e seus benefícios sobre a resposta sistêmica. Já se sabe que a contração do músculo esquelético libera fatores humorais que regulam as funções metabólicas, as chamadas miocinas. As miocinas são citocinas derivadas do músculo esquelético que desempenham

funções fisiológicas e patológicas na manutenção da homeostase, podendo regular o metabolismo de maneira autócrina, parácrina ou endócrina (Goldstein, 1961; So, Kim, Kim e Song, 2014).

Até a década de 2000, sabia-se que a interleucina 6 (IL-6), um importante mediador anti-inflamatório, era produzida em resposta à contração muscular (Fischer, 2006). Entretanto, recentemente foram descobertas novas miocinas que vem sendo amplamente investigadas devido ao seu potencial efeito metabólico. Há, por exemplo, a mionectina que está ligada ao metabolismo de ácidos graxos, o ácido beta-amino-isobutírico (BAIBA) cuja saturação resulta em reduções do tecido adiposo, a secreção de proteínas ácidas e ricas em cisteína (SPARC ou osteonectina) que tem um efeito sobre o metabolismo de carboidratos, a musclina que induz a biogênese mitocondrial e a fibronectina tipo III contendo 5 genes (FNDC5), chamada de irisina (Steensberg et al., 2000; Song et al., 2010; Bostrom et al., 2012; Peterson et al., 2014; Subbotina et al., 2015; Stanford e Goodyear, 2016).

A irisina é um peptídeo formado por 112 aminoácidos clivado de uma proteína de membrana tipo 1 presente em miócitos e é codificada pelo gene FNDC5 (Bostrom, 2012; Hecksteden et al., 2013; Ohtaki, 2016). Especificamente, o FNDC5 é uma proteína formada por um peptídeo sinalizador com 29 aminoácidos, um domínio de fibronectina com 94 aminoácidos e uma terminação carboxila (Bostrom, 2012), possuindo uma similaridade de 100% entre ratos e humanos (Aydin et al., 2014). A irisina é secretada principalmente no músculo esquelético, preferencialmente pelas fibras musculares oxidativas (Bostrom et al., 2012). Recentemente, também foi demonstrado sua secreção pelo pâncreas, músculo cardíaco, glândulas sebáceas e tecido adiposo (Munoz et al., 2018). A liberação de irisina é induzida pelo exercício físico e sua ação periférica promove profundas alterações no tecido adiposo, estimulando fatores intracelulares com funções na biogênese mitocondrial, como por exemplo, na expressão de proteínas desacopladoras mitocondriais (UCP1) (Vaughan et al., 2014; Chen et al., 2015). Alguns estudos visando explicar os mecanismos moleculares da irisina encontraram que o tratamento com a r-irisina (irisina humana purificada) *in vitro* utilizando cultura de adipócitos, aumentou a expressão de UCP1 por meio do aumento da fosforilação da proteína quinase

ativada por mitógeno p38 (MAPK p38) e quinases reguladoras (Zhang et al., 2014). Assim, uma das funções mais importantes da irisina é a regulação da termogênese, devido a sua capacidade de aumentar o gasto energético, promovendo uma perda de massa corporal e redução à resistência à insulina (Bostrom, 2012). Estudos também demostraram que a irisina pode atuar como uma adipocina (Moreno-Naverrete et al., 2013; Roca-Rivada et al., 2013), bem como uma potencial "neurocina" (Huh et al., 2012; Dun et al., 2013).

Embora o papel da irisina no encéfalo ainda não seja claro, estudos demonstraram que a irisina aplicada centralmente aumenta a proliferação de células neuronais do hipocampo de camundongos (Moon et al., 2013). Neste contexto, estudo de Piya et al. (2014) demonstrou a presença de irisina no fluído cérebro espinhal de humanos e receptores para essa miocina no hipotálamo, sugerindo a participação central desse hormônio no controle do metabolismo. Adicionalmente, Dun et al. (2013) reportaram a expressão de irisina e FNDC5 em células de Purkinje do cerebelo de roedores, assim como em neurônios e neuroglia (Dun et al., 2013; Aydin et al., 2014; Gur et al., 2017).

De forma interessante, recentemente foi demonstrado que a irisina também participa do controle cardiovascular (Zhang et al., 2015). Segundo os autores, a irisina atuando no sistema nervoso central (SNC) aumenta o débito cardíaco e a pressão arterial ativando os neurônios do núcleo paraventricular hipotalâmico. Já a irisina periférica derivada do músculo esquelético, em resposta ao exercício ou aos estímulos de frio, pode impedir a elevação da pressão arterial induzida pela ativação simpática (Zhang et al., 2015). Desta forma, os pesquisadores sugerem que a irisina pode atuar como um importante mensageiro entre os órgãos ligando a atividade do músculo esquelético ao encéfalo, tecido adiposo e sistema cardiovascular para integrar o gasto de energia com a atividade cardiovascular. Mais recentemente, o estudo de Gamal et al. (2020) demonstrou que pacientes com artrite reumatóide que apresentavam melhor qualidade de sono, também tinham maiores concentrações de irisina plasmática. Desta forma, os autores sugerem que a irisina pode estar relacionada com a melhora da qualidade do sono em humanos. A expressão e atuação da irisina em vários tecidos demonstra seu papel importante nos diversos sistemas fisiológicos, mas são necessários mais

estudos para confirmar e melhor compreender as funções específicas da irisina, como por exemplo sobre o sistema de controle respiratório (Panati et al., 2016). Como previamente mencionado, não se sabe ainda o que causa o aumento da ventilação durante o exercício físico, uma vez que a $PaCO_2$ e PaO_2 variam minimante. Provavelmente mediadores, como as miocinas, podem fazer parte dessa via de sinalização entre atividade muscular e ajuste da ventilação.

Objetivos

Verificar a participação da irisina atuando no SNC nas respostas ventilatórias, cardiovasculares e metabólicas em condições de normóxia normocápnica, hipóxia e hipercapnia em ratos adultos durante o ciclo sono/vigília.

Revisão da Literatura

Regulação respiratória e Quimiossensibilidade

A respiração dos mamíferos é um processo rítmico que consiste em três fases: inspiração, pós-inspiração e expiração ativa (Ritcher, 1996). Sua principal função é manter relativamente constante a PaO_2 e $PaCO_2$, bem como os valores de pH em todas as condições, seja em exercício, hipóxia, sono ou vigília (Feldman et al., 2003).

O controle respiratório é coordenado por núcleos geradores e integradores do ritmo/padrão respiratório, localizados no sistema nervoso central, especificamente no tronco encefálico (Figura 1). O complexo pré-Bötzinger (preBötC) (Smith et al., 1991; Tan et al., 2008) e o complexo pós-inspiratório (PiCo) (Del Negro et al., 2018; Ramirez e Anderson, 2017) são os responsáveis pela geração da fase de inspiração e pós-inspiração em estado de repouso. Já em condições de alta demanda metabólica, como por exemplo, durante exercício, o núcleo parafacial lateral (pFL), uma subpopulação dentro do grupo respiratório parafacial do núcleo retrotrapezóide (RTN / pFRG) é o

responsável pelo controle da expiração ativa (Del Negro et al., 2018; Ramirez e Anderson, 2017).

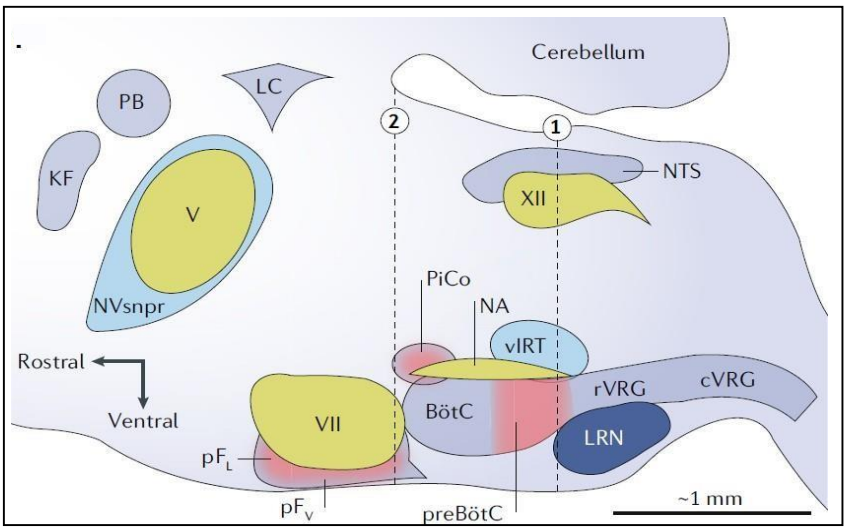

Figura 1: Representação esquemática em corte sagital do tronco encefálico de rato adulto contendo os núcleos geradores de ritmo e padrão respiratório. As regiões ritmogênicas são destacadas em vermelho: pré-BötC (inspiratório); grupo respiratório parafacial lateral (pFL-expiratório) e ventral (pFV- expiratório prevalente na fase perinatal) e complexo pós-inspiratório (PiCo). Regiões moduladoras do padrão respiratório: núcleo do trato solitário (NTS), kölliker-fuse (KF) e parabraquial (PB). Núcleos pré-motores: grupo respiratório ventral rostral (VRGr) e caudal (VRGc) (Del Negro et al., 2018).

Variações na PaO_2 e $PaCO_2$ e nos valores de pH, estimulam sensores especializados para adequar as trocas gasosas e promover o equilíbrio dessas variáveis respiratórias. Existem diferentes sensores que detectam estas variações e atuam na manutenção dos gases sanguíneos e na manutenção do equilíbrio ácido-básico, dentre estes sensores, os principais são os quimiorreceptores periféricos e centrais (Figura 2) (Haldane e Priestley, 1905; Kumar e Prabhakar, 2012). Os quimiorreceptores periféricos estão localizados principalmente no corpo carotídeo, próximo à bifurcação da artéria carótida, e no arco aórtico (Ortega-Sáenz et al., 2013) e são sensíveis a alterações da PaO_2, mas também da $PaCO_2$ e pH, assim como a redução do fluxo sanguíneo e mudanças das concentrações sanguíneas de lactato, potássio e catecolaminas (Kumar e Prabhakar, 2012). Em relação aos quimiorreceptores centrais responsáveis por detectar alterações de CO_2/pH, estes estão espalhados em diferentes regiões do SNC, mas principalmente localizados no

tronco encefálico, e inclui o núcleo do trato solitário (NTS), núcleo retrotrapezóide (RTN) (Mulkey et al., 2004; Guyenet et al., 2009), rafe rostral bulbar (RB), o *locus coeruleus* (LC) (Coates et al.,1993; Nattie, 1999; Ballantyne e Scheid, 2001; Nattie e Li, 2001; Gargaglioni et al., 2010; Nattie e Li, 2010), mas também o núcleo fastigial do cerebelo (NF) e a região perifornical do hipotálamo lateral (Li et al., 2013).

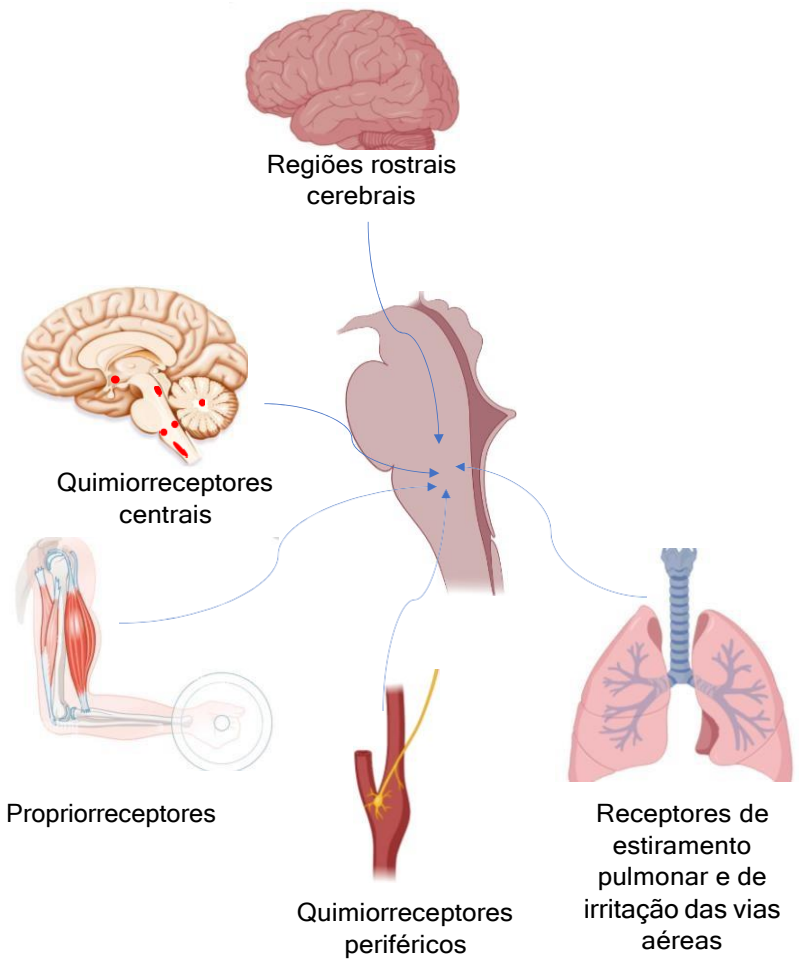

Figura 2: Principais sensores que enviam informações para as regiões de controle respiratório alterando a ventilação.

A participação dos núcleos quimiossensíveis na resposta ao CO_2 ou pH depende de fatores, como o limiar de excitação, anestesia e o estado do ciclo sono-vigília (Nattie 2001; Nattie e Li, 2012). Estudos demonstraram que a quimiorrecepção central está ligada ao estado de sono-vigília de tal modo que algumas regiões são mais importantes durante a vigília e outras durante o sono

(Li et al., 1999; da Silva et al., 2010; Dias et al., 2010; Nattie e Li, 2011; Li et al., 2013; Vicente et al., 2016). Neste contexto, Dias et al. (2009) ao realizar a acidificação focal com 25% CO_2 na região do RTN em animais não anestesiados, observou um aumento da ventilação em vigília, enquanto, ao realizar a acidificação focal com 25% CO_2 na rafe bulbar observou um aumento da ventilação durante o sono (Dias et al., 2010). A realização de diálise com CO_2/H^+ no bulbo ventrolateral, promoveu um aumento da ventilação em animais não anestesiados durante a vigília, mas não durante o sono (Silva et al., 2010). A acidificação local de neurônios orexinérgicos na região perifornical-lateral da área hipotalâmica lateral demonstrou aumentar a ventilação durante o despertar, mas não durante o sono (Li et al., 2013). Também já foi descrito que as projeções de neurônios contendo orexina para o *locus coeruleus* contribuem para o quimiorreflexo hipercápnico durante a vigília na fase escura (Vicente et al., 2016). Esses achados sugerem a estreita relação existente entre a quimiorrecepção central e estado de sono-vigília.

Regulação cardiovascular

A regulação dos gases sanguíneos depende das alterações na ventilação pulmonar, como também dos ajustes no sistema circulatório para ajustar a perfusão tecidual. Essa regulação ocorre por meio de mudanças no débito cardíaco, como também do aumento regulado da pressão arterial e da ativação diferencial do tônus simpático em diversos órgãos (Guyenet et al., 2010).

Os ritmos respiratórios e cardiovasculares são regulados sinergicamente para garantir uma adequada ventilação-perfusão. Esses sistemas são acoplados funcionalmente, com oscilações na pressão arterial, denominadas ondas de Traube-Hering, e na frequência cardíaca, denominada arritmia sinusal respiratória (ASR), que estão em fase com a ciclo respiratório. Esta interação cardiorrespiratória é uma propriedade fisiológica altamente conservada presente em vertebrados, incluindo mamíferos, peixes, anfíbios e répteis (Taylor et al. 1999). Quando dentro da faixa fisiológica, as ondas de Traube-Hering são importantes para as alterações do tônus vascular (Briant et al., 2015), a ASR

reduz o gasto energético cardíaco (Ben-Tal et al., 2012), e acredita-se que ambas otimizam a perfusão do tecido sanguíneo e troca gasosa.

Inúmeros estudos vêm demonstrando que os nervos simpáticos que mantêm a função cardiovascular são modulados pelo gerador do ritmo respiratório central (Gilbey et al., 1984,1996; Haselton e Guyenet, 1989; Habler et al., 1994; Jänig e Häabler, 2003; Mandel e Scheihofer, 2006; Zoccal et al., 2008). Essa interação pode ser evidenciada com base nos critérios funcionais e anatômicos, uma vez que a superfície ventral do bulbo tem sido apontada como o principal local de interações sinápticas entre os neurônios respiratórios e simpáticos. A região do bulbo ventral abriga os neurônios pré-simpáticos do núcleo bulbo ventrolateral rostral (RVLM) que mantêm a pressão arterial basal em níveis adequados (Figura 3) (Guertzenstein e Silver,1974; Ross et al.,1984). Junto aos neurônios RVLM estão os neurônios respiratórios da coluna respiratória ventral (VRC). O VCR é composto por quatro subnúcleos distintos considerados núcleos geradores do ritmo e padrão respiratório: complexo Bötzinger (BötC), complexo pré-Bötzinger (pré-BötC), grupo respiratório ventral rostral (rVRG) e grupo respiratório ventral caudal (cVRG) (Smith et al., 1991 , 2007; Moraes et al., 2011) (Figure 3). Devido à proximidade anatômica destes neurônios, tem se sugerido que esses neurônios respiratórios são responsáveis por estabelecerem conexões sinápticas e gerar a oscilação respiratória sobre a atividade simpática. Neurônios localizados na ponte também demonstraram desempenhar um papel na modulação respiratória da atividade simpática, uma vez que a transecção pontina atenuou significativamente o tônus simpático (Baekey et al., 2008, 2010). De fato, estudo recente demonstrou que os neurônios do pré-BötC têm conexões anatômicas com a região adrenérgica C1 (localizada no RVLM) fornecendo informações da inspiração para esses neurônios pré-simpáticos (Dempsey et al., 2017; Menuet et al., 2017). Também é proposto que a forte inibição inspiratória sobre os neurônios pré-ganglionares vagais cardíacos vem do pré-BötC (Frank et al., 2009). Adicionalmente, há evidências que exposição a hipóxia e hipercapnia promove alterações no acoplamento simpático-respiratório, aumentando as repostas simpáticas durante as atividades motoras inspiratórias e expiratórias (Boczek-Funcke et al., 1992; Braga et al., 2007; Molkov et al., 2011; Moraes et al., 2012).

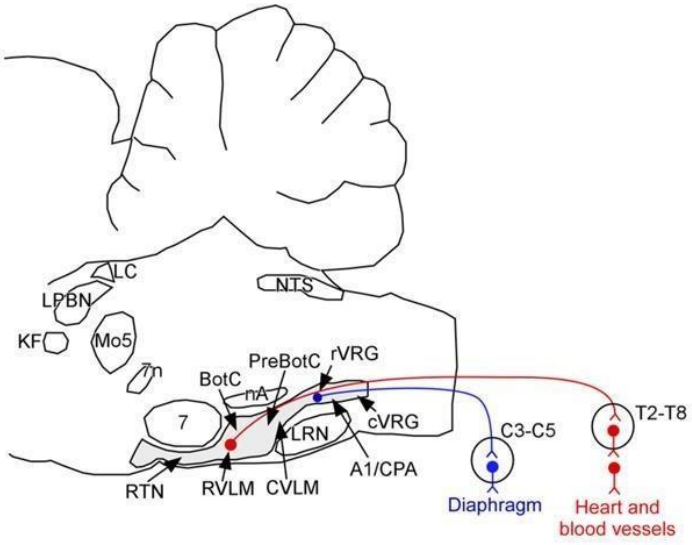

Figura 3: Representação esquemática do tronco encefálico de rato adulto contendo as principais regiões responsáveis pelo controle cardiorrespiratório: núcleo do trato solitário (NTS), bulbo ventrolateral rostral (RVLM) e caudal (CVLM), núcleo retrotrapezóide (RTN), grupo respiratório ventral rostral (rVRG) e caudal (VRGc), complexo pré-Böttzinger (pré-BötC), kölliker-fuse (KF), *locus coeruleus* (LC) (Fonte: Moreira et al., 2011).

Até o momento, é bem aceito que vários mecanismos neurais integrativos trabalhando em conjunto são responsáveis por promover alterações do controle parassimpático e simpático para o coração e vasos sanguíneos, levando a aumentos na FC, débito cardíaco, resistência periférica total e pressão arterial em resposta às contrações musculares (Fisher et al., 2015). Esses mecanismos neurais incluem o comando central, os barorreflexos arteriais e cardiopulmonares, o quimiorreflexo arterial e o reflexo pressor do exercício. De acordo com Fisher et al. (2015), o reflexo pressor do exercício é um mecanismo de feedback negativo que surge do músculo esquelético com fibras aferentes associadas disparando em resposta a estímulos mecânicos e metabólicos durante a contração muscular. Esses sinais aferentes enviam projeções para as áreas de regulação cardiovascular no tronco encefálico que reflexamente diminuem a atividade parassimpática e aumentam a atividade simpática para aumentar o débito cardíaco, a resistência periférica e a pressão arterial. Embora seja amplamente aceito que o reflexo pressor do exercício seja um dos principais mediadores das respostas cardiovasculares ao exercício, as vias dos circuitos

neuronais envolvidos durante a ativação do reflexo pressor do exercício em humanos têm recebido pouca atenção (Teixeira et al., 2020).

Assim como a regulação respiratória, as funções cardiovasculares são moduladas de acordo o estado de sono/vigília (Silvani e Dampsey, 2013; Benarroch, 2019). Em ratos, durante o sono sem movimentos oculares rápidos (NREM), há uma redução progressiva da pressão arterial, que promove uma diminuição da atividade vasomotora simpática e induz um aumento progressivo da modulação parassimpática cardíaca associada à redução da FC (Somers et al., 1993).

Regulação ciclo sono/vigília

Os ciclos diários de vigília e sono são regulados pelos ritmos circadianos. Os ritmos circadianos são ciclos fisiológicos e comportamentais com periodicidade recorrente de aproximadamente 24 horas, gerados pelo marcapasso biológico endógeno, o núcleo supraquiasmático do hipotálamo (SCN) (Golombek e Rosenstein, 2010). Estes ritmos além de controlar o ciclo sono-vigília, são também responsáveis por controlar diversas funções biológicas em sincronia com as mudanças do ciclo claro/escuro como temperatura corporal, alimentação, secreção hormonal, homeostase da glicose e a regulação do ciclo celular (Welsh et al., 2010; Marcheva et al., 2013; Zee et al., 2013)

O eletroencefalograma (EEG) e o eletromiograma (EMG) são excelentes biomarcadores dos estados de sono/vigília (Scammell et al., 2017). No estado de vigília, o cérebro é altamente ativo e o EEG mostra dessincronizado, com baixa amplitude, frequências rápidas, enquanto o EMG mostra quantidades variáveis de atividade muscular, como demonstrado na figura 4. O estado de vigília é regulado por diferentes neurotransmissores e cada um destes são especializados em promover diferentes aspectos da vigília. Por exemplo, a acetilcolina está envolvida nas funções cognitivas, enquanto a noradrenalina e a dopamina estão envolvidas em experiências salientes, como novidade e estresse, além de estar envolvido em funções cognitivas (Ballinger et al., 2016; Borodovitsyna et al., 2017; Cho et al., 2017; Oishi. e Lazarus, et al., 2017;

Arrigoni et al., 2019). Já a orexina é responsável tanto pela vigília consolidada como também está envolvida em comportamentos motivados, como comer (Arrigoni et al., 2019).

Durante o sono NREM, o EEG é dominado por frequências mais lentas com altas amplitudes e o EMG mostra baixa atividade muscular (Figura 4). Estudos demonstram que que a área pré-óptica ventrolateral (VLPO) e o núcleo pré-óptico mediano (MnPO) contêm neurônios essenciais para promover o sono NREM (Alam et al., 2014 ; Sherin et al., 1996). A VLPO contém neurônios GABAérgicos/galaninérgicos que estão ativos durante o sono (Szymusiak et al., 1998; Takahashi et al., 2009) e se projetam e inibem os sistemas promotores da vigília (Sherin et al., 1998). Estudos já relataram que a ativação dos neurônios galaninérgicos na VLPO aumentam o sono NREM ao mesmo tempo em que atenuam significativamente a temperatura corporal (Yoshida et al., 2009).

O sono de movimento rápido dos olhos (REM) é caracterizado por apresentar um EEG semelhante ao descrito durante o estado de vigília (Figura 4), podendo ser distinguindo devido à presença de fenômenos fásicos como por exemplo, o movimento rápido dos olhos e espasmos dos membros e neste estado e o EMG mostra totalmente atonia muscular (Gompf e Anaclet, 2020). O circuito central que gera o sono REM está localizado no tronco cerebral, sendo já descrito a presença de neurônios que regulam o sono REM, em todo o bulbo, ponte, mesencéfalo e hipotálamo (Park e Weber, 2020).

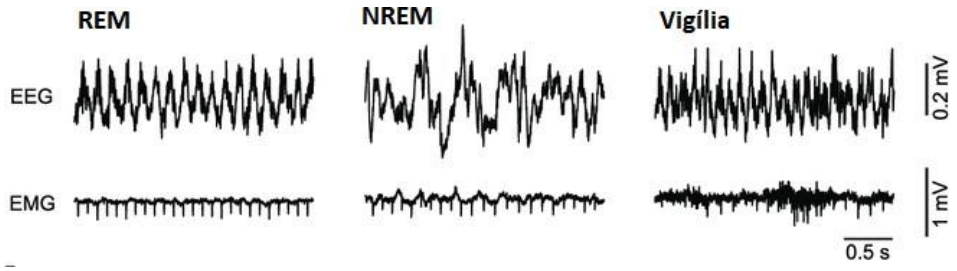

Figura 4: Registro de eletroencefalograma (EEG) e o eletromiograma (EMG) de um camundongo durante o sono REM, sono NREM e vigília. (Figura adaptada de Park e Weber, 2020).

Miocinas

Estudos têm demonstrado que o músculo esquelético não é apenas uma unidade locomotora pura ou responsável por propulsão e postura. Este já é reconhecido como um órgão capaz de produzir e secretar moléculas que exercem diversas funções fisiológicas na manutenção da homeostase sistêmica (Pedersen e Febbraio,2012; Pedersen, 2013; Giudice e Taylor, 2017). Essas moléculas são denominadas de miocinas e ao serem produzidas regulam as funções metabólicas do organismo de maneira autócrina, parácrina e endócrina (So et al., 2014).

Dessa forma, ao serem produzidas pelas fibras musculares as miocinas podem interagir com outros órgãos e regular diferentes funções (Huh, 2018). Diversas miocinas já foram descritas e o seu papel como molécula sinalizadora já foi relatado devido a sua interação com receptores localizados nos músculos, ossos, fígado, encéfalo, coração, tecido adiposo, rins e pâncreas. Por exemplo, a IL-6 e a miostatina podem atuar regulando o crescimento e desenvolvimento do músculo esquelético (Pedersen e Febbraio, 2008; Allen et al., 2011). Enquanto o fator de crescimento semelhante à insulina 1 (IGF-1) e o fator de crescimento de fibroblastos-2 (FGF-2) participam da formação e reparo ósseo (Locatelli e Bianchi, 2014; Yan et al., 2016; Lkpegbu et al., 2018). Estudos já demonstraram que a irisina promove a proliferação e diferenciação de osteoblastos (Colaianni et al., 2016; Ma et al., 2018). A IL-6 (Fritsche et al., 2010) também contribui para a homeostase hepática e metabólica assim como a mionectina (Seldin et al., 2012), o BAIBA (Shi et al., 2016), o fator de crescimento de fibroblastos 21 (FGF21) (Liu et al., 2018) e a irisina (Xin et al., 2016). A catepsina B (CTSB) (Pedersen, 2019), o fator neutrófico derivado do cérebro (BNDF) (Brailoiu et al., 2015; Ruan et al., 2018) e a irisina (Hashemi et al., 2013; Moon et al., 2013) são alguns exemplos de miocinas descritas desempenhando funções no encéfalo. Entre as miocinas induzidas pelo músculo que foram relatadas atuando em funções cardiovasculares estão as: dermicidina (Esposito et al., 2015), folistatina 1 (FSTL1) (Norheim et al., 2011; Gorgens et al., 2013; Miyabe et al., 2014), mionectina (Otaka et al., 2018), apelina (Parikh et al., 2018), musclina (Li et al., 2013; Lin et al., 2014) e a irisina (Zhang et al., 2015; Yu et al.,

2019; Huo et al., 2020). Destaca-se a contribuição da irisina na regulação do metabolismo lipídico nos adipócitos (Bostrom et al., 2012).

De acordo com as informações supracitadas, a irisina é uma miocina que pode atuar em diversos órgãos modulando múltiplas respostas no organismo. A síntese e a liberação de irisina é regulada principalmente pelo exercício físico (Kelly, 2012). Neste contexto, Bostrom et al. (2012) identificaram que durante a atividade física, a expressão do co-ativador-1α do receptor γ ativado por proliferador de peroxisoma (PGC-1α) no músculo esquelético desencadeia um aumento na expressão de FNDC5, uma fibronectina tipo III, localizada nas membranas de células musculares que leva a produção de irisina. Nas células musculares, a contração gerada pelos músculos promove uma ativação das vias de sinalização por AMPK (proteína quinase ativada por adenosina monofosfato) ou p38MAPK (p38 proteína quinase ativada por mitógeno) que estimulam a expressão de PGC-1α. O aumento do PGC-1α na célula impulsiona o núcleo à um processo de transcrição que dará origem ao FNDC5. A produção da irisina ocorre a partir de uma clivagem enzimática do FNDC5 feita pela enzima da família desintegrina e metaloproteinase (Yu et al., 2019; Rabbie et al., 2020; Fu et al., 2021) (Figura 5).

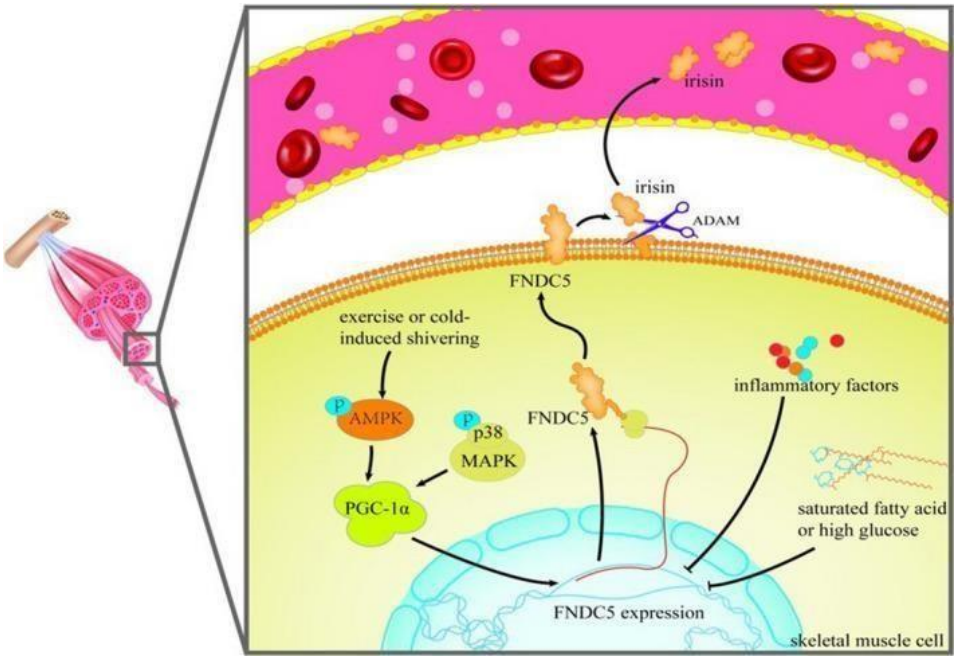

Figura 5: Representação esquemática da produção de irisina nas células musculares – desintegrina e metaloproteinase (ADAM), proteína quinase ativada por monofosfato de adenosina (AMPK), domínio de fibronectina do tipo III contendo 5 (FNDC5), proteína quinase ativada por mitógeno (MAPK), co-ativador-1α do receptor ativado por proliferador (PGC-1α) (Fu et al., 2021).

A primeira função fisiológica da irisina descrita foi o seu papel na conversão do tecido adiposo branco (TAB) em tecido adiposo marrom (TAM) (processo denominado *"Browning"*) promovendo gasto de energia e auxiliando na redução da massa corpórea (Bostrom et al., 2012; Saely et al., 2012; Elattar e Satyanarayana 2015). Sabe-se que o TAM é abundante em roedores e ocorre em menor quantidade em grandes mamíferos, estando presente em humanos durante a vida intrauterina e em lactentes nas regiões interescapular e perirrenal, mas mesmo essas pequenas quantidades desaparecem gradualmente (Heaton, 1972). Em adultos, o TAM é remanescente e sem atividade fisiológica significativa (Cypess et al., 2009). No entanto, a recente descoberta de TAM ativo em humanos adultos e a documentação de vários fatores de transcrição que regulam a formação de novos adipócitos termogênicos faz com que seja atrativo aumentar esse tipo de tecido adiposo e utilizá-lo como alvo terapêutico (Harms e Seale, 2013). A gordura subcutânea pode ficar "marrom" sob vários estímulos, como frio, agonistas beta-adrenérgicos ou estímulos semelhantes a hormônios (Vitali et al., 2012) (Figura 6).

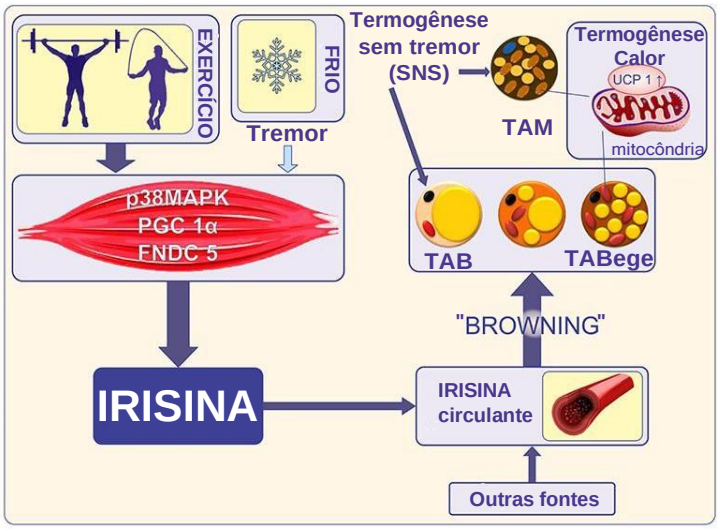

Figura 6: Representação esquemática dos fatores de indução da produção e liberação de irisina (atividade física e exposição ao frio) e suas regiões alvo como tecido adiposo branco (TAB) e bege (TABege) no mecanismo de termogênese vinculados a ativação mitocondrial de unidades desacopladoras (UCP1). Abreviações: TAM: tecido adiposo marrom; UCP1: proteína desaclopadora de prótons tipo 1; SNS: sistema nervoso simpático. (Figura adaptada de Arhire et al., 2019)

Como ilustrado na Figura 6, a exposição ao frio estimula os eventos de tremores no músculo esquelético, bem como uma fase sem tremor na qual o tecido adiposo marrom (TAM) é ativado via sistema simpático para produção e liberação de calor via UCP1 mitocondrial. Adicionalmente, tremores e atividade física promovem a termogênese mediada pelo tecido adiposo através da secreção de irisina. O exercício aumenta o coativador transcricional PGC1-α e induz a expressão do gene FNDC5. A proteína de membrana FNDC5 é clivada para liberar irisina na corrente sanguínea. A irisina também pode ser liberada pelos adipócitos, tornando-se uma adipocitocina (Lee et al., 2014). Outras fontes, como encéfalo, pâncreas, estômago, células de Kupffer, língua, coração, testículos, células epiteliais sinusoidais e nervo óptico também são capazes de liberar irisina (Aydin et al., 2014). Desse modo, a irisina atua no tecido adiposo promovendo o “escurecimento” dos adipócitos brancos maduros em resposta ao exercício (Bostrom et al., 2012; Fox et al., 2018). Por sua vez, o TAM e TABege aumentam o gasto energético por meio do desacoplamento do metabolismo oxidativo da produção de ATP pelas mitocôndrias, sendo uma função chave da UCP1 (Sharp et al., 2012). De modo sinérgico, a irisina possui um papel

importante relacionado com a regulação do metabolismo da glicose (Perakakis et al., 2017).

Sobre a atuação da irisina no SNC, já foi descrito que a mesma pode atravessar a barreira hematoencefálica (Zhang et al., 2018), mas também ser secretada *in situ* pelas células de Purkinje no córtex cerebelar de roedores, assim como em neurônios e neuroglia (Dun et al., 2013; Aydin et al., 2014; Gur et al., 2017). Estudos subsequentes revelaram que o aumento da secreção de irisina promove a liberação do fator neurotrófico derivado do cérebro (BDNF) no hipocampo (Wrann et al., 2013). Uma vez que o BDNF está intimamente relacionado com a doença de Alzheimer (Budni et al., 2012), alguns autores buscaram avaliar o papel da irisina relacionado a essa fisiopatologia. Os estudos evidenciaram que a irisina secretada pelo encéfalo pode prevenir a progressão desta doença (Azimi et al., 2018; Lourenço et al., 2019).

Alguns estudos também já relataram o papel da irisina no controle cardiovascular. O estudo de Zhang et al. (2015) demonstrou que a administração de irisina no núcleo paraventricular do hipotálamo aumentou a pressão arterial e a contratilidade cardíaca, já a administração periférica de irisina reduziu a pressão arterial em ratos controle e espontaneamente hipertensos. Por outro lado, foi relatado que a microinjeção de irisina no núcleo ambíguo promoveu uma redução significativa da FC de ratos (Brailoiu et al, 2015). A injeção intravenosa de irisina resultou em redução da pressão arterial, da norepinefrina plasmática e da ativação neuronal do núcleo paraventricular do hipotálamo, bem como reduziu o estresse oxidativo e a inflamação em ratos espontaneamente hipertensos (Huo et al., 2020).

Estudos evidenciaram que a irisina também pode estar relacionada com a melhora da qualidade do sono (Gamal et al. 2020; Tan et al., 2020). No entanto, ainda há uma escassez de estudos sobre o papel da irisina no controle das funções fisiológicas, tal como a participação da irisina central nas respostas ventilatórias, cardiovasculares e metabólicas frente a hipercapnia e hipóxia. Diante disso, o objetivo do presente estudo foi verificar a participação central da irisina nas respostas cardiorrespiratórias e metabólicas em condições de normóxia normocápnica, hipercapnia e hipóxia em ratos adultos durante o ciclo sono/vigília.

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CAPÍTULO 2 - PARTICIPATION OF CENTRAL IRISIN IN CARDIORESPIRATORY AND METABOLIC CONTROL OF ADULT RATS

Abstract: Irisin is a newly identified hormone consisting of 112 amino acids, derived from a transmembrane protein encoded by the type III fibronectin gene containing 5 (FNDC5), which is highly related to the metabolic activity of skeletal muscle and adipose tissue, having a similarity of 100 % between rats and humans. It is known that the concentration of irisin increases during physical exercise, bringing beneficial effects to various tissues, including the central nervous system, promoting neuroplasticity, since irisin seems to be associated with neural proliferation and differentiation. It has also recently been shown that this hormone modulates cardiovascular function, possibly acting as an important messenger between organs to integrate energy expenditure with cardiovascular activity. Therefore, the present study evaluated the effect of intracerebroventricular (icv) injection of vehicle or irisin (IR, 0.425 or 1.66 μg/μL) in the fourth ventricle (4V) of adult male rats in ventilation ($\dot{V}_E$), oxygen consumption ($\dot{V}O_2$), ventilatory equivalent ($\dot{V}_E / \dot{V}O_2$), body temperature (T_B), mean arterial pressure (MAP) and heart rate (HR) in wakefulness and sleep state during room air, hypercapnia (7% CO_2) and hypoxia (10% O_2). Treatment with irisin centrally with the lowest concentration (0.425 μg/μL), promoted a reduction in $\dot{V}_E$ in room air conditions during wakefulness, culminating in hypoventilation (lower $\dot{V}_E / \dot{V}O_2$). During exposure to room air conditions, animals treated with the highest concentration of irisin (1.66 μg/μL) also showed a reduction in $\dot{V}_E$ and f_R. The animals treated with the highest concentration of irisin when exposed to high levels of CO_2 showed an increase in $\dot{V}_E$, due to a higher V_T and a reduction in $\dot{V}O_2$, which resulted in hyperventilation (higher $\dot{V}_E / \dot{V}O_2$) during wakefulness. The both concentrations of irisin attenuated hypoxia-induced regulated hypothermia. Regarding cardiovascular parameters, central irisin injection with the lower concentration caused a significant increase in HR under all conditions during sleep and wakefulness, without altering MAP. The treatment with the highest concentration promoted an increase in HR in the animals when exposed to hypoxia during sleep and wakefulness states and during hypercapnia in sleep state. Our data demonstrate that irisin exerts an inhibitory effect on breathing control and potentiates the hyperventilatory response under hypercapnia, promoting an increase in $\dot{V}_E$ and inhibiting $\dot{V}O_2$ during wakefulness. In addition, this myokine is excitatory to HR and attenuates the drop in body temperature in low O_2 conditions.

Keywords: Irisin, ventilation, metabolism, myokines

Introduction

The principal function of respiration is to maintain the arterial partial pressure of oxygen (PaO_2) and arterial partial pressure of carbon dioxide ($PaCO_2$) relatively constant, as well as the pH values. This process is highly integrated and involves a complex network of interaction composed by the brain, spinal cord, cranial and spinal nerves, specific musculature and lungs. In addition, this process depends on structural and functional changes of neurotransmitters, neuromodulators and receptors (Anju et al., 2011; Wong-Rileyet al., 2013; Del Negro et al., 2018).

In humans, during conditions of light or moderate physical exercise, partial pressures of blood gases change minimally, despite of the increase in metabolic rate as a result of physical exertion (Dempsey and Rankin, 1967; Forster et al., 1986). Therefore, for more than a century there has been intense interest in the elucidation of how respiratory neurons adjust their activity specially in response to physical exercise since chemoreceptors seems not to be involved in the exercise-induced hyperpnea (Forster et al., 2012).

It is widely known that skeletal muscle contraction releases humoral factors to regulate metabolic functions, the called myokines. Myokines are cytokines derived from skeletal muscle that play physiological and pathological roles in the maintenance of systemic homeostasis (Goldstein, 1961; So et al., 2014). Myokines are involved in the autocrine regulation for muscle metabolism, whereas in other tissues and organs such as adipose tissue, the brain, and the liver, they are responsible for paracrine or endocrine regulation through their receptors (Carson, 2017). Irisin is a myokine composed by 112 amino acids, derived from a type 1 membrane protein present in myocytes and is encoded by the fibronectin type III domain gene containing 5 (FNDC5) (Bostrom, 2012; Hecksteden et al., 2013; Ohtaki, 2016). More specifically, FNDC5 results in a protein formed by a signal peptide with 29 amino acids and has a 100 % similarity between rats and humans (Aydinet al., 2014). Previous studies show that irisin converts white adipose tissue into brown adipose tissue mainly by upregulating the uncoupling protein 1 (UCP1) expression through enhanced energy expenditure (Bostrom et al., 2012). Since then, multiple other functions have been

described such as irisin stimulates neuroplasticity in the brain (Moon et al., 2013), participation in cardiovascular control (Zhang et al., 2015) and effects on sleep quality (Gamal et al., 2020; Tan et al., 2020).

As previously mentioned, arterial blood gas partial pressures vary minimally during exercise (Forster et al., 2012). Probably other mediators, such as irisin, may be part of this signalling pathway between muscle activity and respiratory rhythm adjustments. Therefore, the goal of the present study was to verify the participation of central irisin in the cardiorespiratory regulation under room air conditions, hypoxia and hypercapnia in adult male rats during sleep and wakefulness.

Methods

Animals

The experiments were performed with adult male Wistar rats, weighting around 300 to 350 g. All the animals were housed in a temperature-controlled room, maintained at 25 ± 1°C with a 12 h light-dark cycle (lights on at 6:30 a.m.), with water and food provided *ad libitum*. Experiments were performed between 7:00 a.m. and 6:00 p.m at the Department of Animal Morphology and Physiology of the Faculty of Agricultural and Veterinary Sciences – FCAV/UNESP, campus of Jaboticabal.

All experimental protocols were approved by the local ethics committee (CEUA - protocol n° 3337/20), being in norm with the National Council for the Control of Animal Experimentation (CONCEA-MTC, Brazil).

Irisin treatment and gas mixture exposure

The irisin from Cayman Chemical (Ann Arbor, MI – EUA) was diluted in tris solution buffer (0.05M, pH ~8). This solution was used as vehicle for the control animals. We tested two concentrations of irisin, 0.425 and 1.66 µg/µL (based on previous study of Zhang et al., 2015). The ventilatory challenges were accomplished using gas mixture of hypercapnia (7% CO_2, 21% O_2, balance N_2)

and hypoxia (10% O_2, balance N_2) purchased from White Martins Gases Industrials Ltda (Osasco, SP, Brazil).

Implantation of guide cannula in the 4th ventricle, electromyogram (EMG) and electroencephalogram (EEG)

One week prior to the experimental day, the rats were anesthetized with intraperitoneal injection of ketamine and xylazine (100 e 10 mg/kg, respectively). The animals were fixed in a stereotaxic apparatus (Kopf Instruments, Kent, England) and a stainless-steel guide cannula (15 mm in length and 0.7 mm in external diameter) was implanted in the fourth ventricle according to the coordinates adapted from Paxinos and Watson atlas (1998) (AP: -11.9 mm from the bregma, LL: 0 mm, DV: -6.7 mm from the skull). The cannula was fixed to the skull using dental acrylic and sealed with a tight-fitting stylet to prevent occlusion and infection.

After this procedure, three EEG electrodes were screwed into the skullcap: first electrode: 2 mm anterior to bregma and 2 mm lateral to the midline; second electrode: 4 mm anterior to the lambda and 2 mm lateral to the midline; third electrode: laterally between the first and second electrodes forming a triangle. For EMG recordings, two electrodes were inserted deep into the neck musculature of the rats. All the electrodes were fixed to the animal's head using a mini connector, that was also soaked in acrylic cement. At the end, the animals were treated with antibiotic (enrofloxacin, 10 mg/kg, subcutaneous) and analgesic (flunixin meglumine, 2.5 mg/kg, subcutaneous) agents followed by the next 2 days and kept in cages up to two animals.

The signals from the EEG and EMG electrodes were sampled at 150 Hz, filtered at 0.3–50 and 0.1–100 Hz, respectively, and recorded on a computer with a data analysis software (AcqKnowledge MP150, BioPac Systems, Inc., Santa Barbara, CA, USA). Wakefulness, rapid eye movement (REM) or non-rapid eye movement (NREM) sleep states were registered constantly throughout the experiments. REM sleep state were short and occurred irregularly between the experiments; thus, REM sleep periods were excluded from analysis. The sleep/wake state was determined by analyzing the EEG and EMG records as

previously described (Nattie and Li, 2002; Vicente et al., 2016; Leirão et al., 2017), allowed cardiorespiratory and metabolic parameters analyses during different phases of the sleep/wake cycle.

Cardiovascular and body temperature measures

One day before the experiment, the rats received an intraperitoneal injection of ketamine and xylazine for the surgical procedure. This surgery consisted of insertion of a catheter [PE-10 connected to PE-50 (Clay Adams, Parsippany, NJ, USA)] into the abdominal aorta through the femoral artery, to allow pulsatile arterial pressure (PAP) and blood samples. The catheter was externalized in the animal's dorse close to the neck region. On the experiment day, the catheter was connected to the pressure transducer (TSD 104A, Biopac systems), the signal was amplified (DA 100C, Biopac systems) anddigitized on a computer equipped with data acquisition software (MP100ACE; Biopac Systems). Systolic (SAP) and diastolic (DAP) arterial pressure, mean arterial pressure (MAP) and heart rate (HR) were quantified from the PAP using the same system (MP100 A-CE, Biopac systems, Inc, Santa Barbara, CA).

For body temperature (T_B) measurements, a temperature datalogger (SubCue Dataloggers, Calgary, Canada) was inserted into the abdominal cavity through a midline laparotomy, at the same surgical procedure. The datalogger was programmed to acquire data every 5 min.

Central microinjection in 4th ventricle

For intracerebroventricular microinjection (icv), a Hamilton syringe (5 µL) and a needle (Mizzy 200 µm of external diameter) where the tip was connected to a PE-10 polyethylene tube that connected to a 30 G gingival needle (15.5 mm length), was used to perform microinjections in the 4th ventricle. The volume of injections were 1 µL during 1 minute using a microinjection machine (model 310, StoeltingCo., Il, EUA).

Pulmonary ventilation assessment

The pulmonary ventilation ($\dot{V}_E$) of rats were acquired by whole body plethysmography – closed system. For $\dot{V}_E$ measurements, the chamber was fully sealed for approximately 2 min. The pressure oscillation due to temperature difference from inhaled and exhaled air were monitored by a differential pressure transducer (TSD 160A, Biopac Systems, Santa Barbara, CA, USA) and fed into a pre-amplifier (DA 100C,Biopac Systems), passed through an analog-to-digital converter and digitized ona computer equipped with data acquisition software (MP100ACE, Biopac Systems). The sampling frequency was 200 Hz. A volume calibration wasperformed by injecting 1 mL of air into the chamber using a graduated syringe. Tidal volume (V_T) was calculated using Drorbaugh and Fenn's formula (1955):

$$V_T = V_K \times (P_T/P_K) \times T_B \times (P_B - P_C) / T_B \times (P_B - P_C) - T_A \times (P_B - PR)$$

where P_T: pressure deflection associated with each V_T, P_K: pressure deflection associated with the injection of the calibration volume (V_K), T_A: air temperature in the animal chamber, P_B: barometric pressure, P_C: water vapor pressure in the animal chamber, T_B: body temperature (in Kelvin), and P_R: water vapor pressure at T_C.

The $\dot{V}_E$ was calculated as the product of respiratory frequency (f_R) and V_T and normalized to the animal's body weight. $\dot{V}_E$ and V_T were presented under ambient barometric pressure conditions at T_B and saturated with water vapor (BTPS). P_C and P_R were calculated indirectly using an appropriate table (Dejours, 1981). The LabChart software (PowerLab System, ADInstruments®/LabChart Software, version 7.3, Sydney, Australia) was used for data analysis.

Determination of oxygen consumption

The indirect calorimetry method by measuring O_2 consumption ($\dot{V}O_2$) with flow through push mode configuration in an open respirometry system was used for metabolic rate inference. The chamber air flow (2.2 $L.min^{-1}$) was maintained through a compressor pump that directed the air to the chamber entrance when the animals were exposed to room air and by gas mixture cylinders coupled to a flowmeter when exposed to hypercapnia or hypoxia. The expired gas was dried over a small column of Drierite (W.A. Hammond Drierite Co. Ltd, Xenia, OH, USA) before goes through the gas analyzer. The air was continuously sampled allowing for the determination of $\dot{V}O_2$ by a data acquisition program (Power-Lab System, ADInstruments® / Chart Software, version 7.3, Sydney, Australia).

The CO_2 was not analysed or scrubbed, then aiming at a better metabolic rate measurement the $\dot{V}O_2$ was calculated using the following equation (Koteja, 1996):

$$\dot{V}O_2 = [FR_e (F_iO_2 - F_eO_2)] / [1 - F_iO_2 (1 - RQ)]$$

where FR_e: end flow rate of air through the chamber, F_iO_2: inlet O_2 fraction, F_eO_2: end O_2 fraction, and RQ: respiratory quotient (considered to be 0.85). The $\dot{V}O_2$ was divided for the body mass and values were presented as STPD (standard conditions of temperature, pressure and dry air).

Blood gases and pH measurements

Ninety microliters of blood were sampled from i.c.v - Irisin and vehicle animals during room air, hypercapnia and hypoxia conditions for immediate analyses of arterial pH (pHa), arterial carbon dioxide partial pressure ($PaCO_2$), arterial oxygen partial pressure (PaO_2) and plasma bicarbonate (HCO_3^-). The samples were transferred to an $EG8^+$ cartridge to be read in an i-STAT blood gas portable analyzer (i-STAT Analyzer, Abbott Laboratories, Libertyville Township, IL, USA).

Experimental protocol

The animals were previously placed inside of a plethysmography chamber and the T_B was continuously measured using data loggers. Initially, the chamber was ventilated with atmospheric air (21% O_2), during habituation time of 60 min. Next, the basal $\dot{V}_E$, $\dot{V}O_2$ e PAP were measured in room air throughout 60 min. Following, the animals received an i.c.v injection of irisin (0.425 or 1.66 µg/µL) or vehicle. After injection, the animals were exposed to atmospheric air (21% O_2), hypercapnia (7% CO_2) or hypoxia (10% O_2) during 60 min. For every condition of exposure different animals were used.

During the 60 min of exposure, the cardiorespiratory measures were made every 10 min. The blood sample was collected previously and 15 min after the microinjection. The $\dot{V}O_2$, PAP, T_B measurements and sleep/wake cycle signals were recorded throughout the experiment.

Confirmation of the microinjection site

To identify central microinjection site, at the end of the experiments the animals were deeply anesthetized and received a microinjection of 1 µL of Evans blue via guide cannula. The animals were perfused through the left ventricle of the heart with 60 mL of sterile saline followed by 60 mL of 10% formalin. Then, the animals were decapitated, the brain was removed and sectioned for identification and confirmation of the microinjection site.

Data analyses

The $\dot{V}_E$, $\dot{V}O_2$, MAP, HR e T_B of control and treated animals were grouped according to sleep or wakefulness state in each exposure condition. A mean of the variables was obtained for each state of the sleep-wake cycle. Approximately 1 min of recording was used to measure $\dot{V}_E$ and 2 min for the $\dot{V}O_2$.

The cardiorespiratory, metabolic and thermal variables were compared between treatments groups (vehicle and irisin) within each exposure condition by one-way ANOVA, followed by Tukey's post hoc test. Blood gases and pH data

were compared between treatment groups and gas conditions using two-way ANOVA. P<0.05 was considered statistically significant.

Results

Wakefulness state

Cardiorespiratory, metabolic and body temperature variables of animals in resting conditions during wakefulness prior to microinjection of vehicle or irisin

Table 1 shows the $\dot{V}_E$, V_T, f_R, $\dot{V}O_2$, $\dot{V}_E/\dot{V}O_2$, MAP, HR and T_B data of animals during the resting conditions in wakefulness state before vehicle or irisin (0.425 and 1.66 μg/μL) microinjection. No difference was found between the groups.

Effect of central irisin microinjection on ventilation, metabolism, cardiovascular variables and body temperature under room air, hypercapnic and hypoxic conditions during wakefulness

Figure 1 shows $\dot{V}_E$, V_T, f_R, $\dot{V}O_2$, $\dot{V}_E/\dot{V}O_2$ and T_B of control and irisin treated-animals (0.425 and 1.66 μg/μL) during normoxia normocapnia in the wakefulness. The treatment with two concentrations of irisin promoted a significant reduction in $\dot{V}_E$ under room air conditions (P=0.02 for both concentrations, one-way ANOVA), and the treatment with the highest concentration also reduced the f_R (P=0.02, one-way ANOVA).

The $\dot{V}O_2$ of the animals did not change with the treatments, however, a reduction was observed in the $\dot{V}_E/\dot{V}O_2$ of the animals that received the lowest irisin concentration compared to control and animals treated with the highest concentration (P=0.04 and P=0.03, one-way ANOVA, respectively).

Figure 2 presents $\dot{V}_E$, V_T, f_R, $\dot{V}O_2$, $\dot{V}_E/\dot{V}O_2$ and T_B of control and irisin treated-animals during hypercapnia in the waking state. Hypercapnia induced an

increase in $\dot{V}_E$ by causing tachypnea and hyperpnea. The central microinjection of the highest irisin concentration promoted an increase in $\dot{V}_E$ (P<0.05, one-way ANOVA) compared to the control animals. The effect was due to a significant change in V_T (P<0.01 for control and P=0.01 for IR 0.425 µg/µL; one-way ANOVA). The highest irisin concentration promoted a reduction in $\dot{V}O_2$ compared to control and lowest concentration group (P=0.07, one-way ANOVA, for both groups), as well as an increase in $\dot{V}_E / \dot{V}O_2$ (P<0.03 and P<0.02, one-way ANOVA, respectively) leading to a hyperventilation.

The $\dot{V}_E$, V_T, f_R, $\dot{V}O_2$, $\dot{V}_E / \dot{V}O_2$ and T_B of control and irisin treated-animals during hypoxia in the awake state are shown in the Figure 3. Hypoxia enhanced $\dot{V}_E$, mainly acting on f_R, without any differences between the groups. No change in $\dot{V}O_2$ was observed during low O_2 conditions, but an increase in $\dot{V}_E / \dot{V}O_2$ induced by $\dot{V}_E$ alteration was observed with no difference among the groups. Hypoxia caused a drop in T_B in the vehicle group, which was not observed in the animals that received both irisin concentrations (P<0.05, one-way ANOVA, for both treatments).

Regarding the cardiac variables, Figure 4 represents the values of mean arterial pressure (MAP) and heart rate (HR) of animals treated with irisin (0.425 or 1.66 µg/µL) or vehicle during resting conditions (A), hypercapnia (B) and hypoxia (C) in wakefulness. Treatment with the lowest irisin concentration promoted an increase in HR under all conditions. During room air, this increase was different compared to control and animals treated with the highest concentration (P<0.02, one-way ANOVA, for both groups). In hypercapnic conditions, the microinjection of the lowest irisin concentration also resulted in a significant increase in HR in comparison to the control group (P<0.03, one-way ANOVA). During hypoxia, treatment with both irisin concentrations promoted an increase in HR compared to the control animals (P=0.04, one-way ANOVA, for both treatments).

Sleep state

Effect of central irisin microinjection on ventilation, metabolism, cardiovascular variables and body temperature under resting, hypercapnic and hypoxic conditions during sleep

Figure 5 shows $\dot{V}_E$, V_T, f_R, $\dot{V}O_2$, $\dot{V}_E/\dot{V}O_2$ and T_B of control and irisin treated animals during normoxia normocapnia in sleep state. Treatment with the highest irisin concentration promoted a reduction in f_R, differing from control and animals treated with the lowest concentration (P<0.04, one-way ANOVA, for both groups). Additionally, the treatment with the highest concentration caused a reduction in $\dot{V}O_2$ (P=0.02, one-way ANOVA) compared to the lowest concentration and an increase in $\dot{V}_E / \dot{V}O_2$ compared to control and lowest concentration groups (P<0.03 and P=0.03, one-way ANOVA, respectively).

The values of $\dot{V}_E$, V_T, f_R, $\dot{V}O_2$, $\dot{V}_E / \dot{V}O_2$ and T_B of control and irisin treated rats during hypercapnia in sleep state are demonstrated in Figure 6. Hypercapnia promoted an increase in $\dot{V}_E$, by tachypnoea and hyperpnea. CO_2 exposure did not change $\dot{V}O_2$, but promoted hyperventilation (increase in $\dot{V}_E / \dot{V}O_2$) by changing the $\dot{V}_E$. No significant changes were found in the ventilatory, metabolic and T_B variables during sleep state between control and treated animals.

Figure 7 presents the values of $\dot{V}_E$, V_T, f_R, $\dot{V}O_2$, $\dot{V}_E/\dot{V}O_2$ and T_B of control and irisin treated animals during hypoxia in sleep state. Hypoxia promoted an increase in $\dot{V}_E$, acting on V_T and f_R, with no difference among the groups. The drop in PO_2 promoted hyperventilation (increase in $\dot{V}_E/\dot{V}O_2$) due to changes in $\dot{V}_E$ that were similar between the groups. Hypoxia caused a drop in T_B in the vehicle group, which was not observed in animals treated with both irisin concentrations (P<0.02, one-way ANOVA, for both treatments).

Regarding MAP and HR, treatment with the lowest irisin concentration promoted a significant increase in HR in all exposure conditions. During normoxia normocapnia, this increase differed between control and animals treated with the highest concentration (P<0.03 and P<0.001, one-way ANOVA; respectively). In hypercapnic and hypoxic conditions, both irisin concentrations caused a

significant increase in HR compared to the control group (P<0.02, one-way ANOVA, for both concentrations and conditions).

Effect of central microinjection of irisin on arterial pH and blood gases

Table 2 shows the effect of irisin (0.425 or 1.66 µg/µL) microinjection on arterial pH (pHa), arterial partial pressure of oxygen (PaO_2), arterial partial pressure of carbon dioxide ($PaCO_2$) and plasma bicarbonate (HCO_3^-) during room air conditions, hypercapnia and hypoxia. For all groups, exposure to 7% CO_2 resulted in a decrease in pHa, increases in $PaCO_2$, PaO_2 and HCO_3^- compared to room air conditions and hypoxia (P<0.001, two-way ANOVA, for all variables and conditions). Low environmental O_2 condition caused an increase in pHa and a decrease in $PaCO_2$ and PaO_2 when compared to normoxia (P<0.001, two-way ANOVA, for all variables). Additionally, the animals treated with the highest irisin concentration showed a significant increase in pHa (P=0.03, two-way ANOVA) during room air conditions and a lower $PaCO_2$ during hypercapnia (P=0.01, two-way ANOVA), as well as a reduction in HCO_3^- (P<0.02, two-way ANOVA) when compared to the control group.

Discussion

The data of the current study shows the effects of centrally injected irisin on the cardiorespiratory and metabolic control of Wistar adult rats during sleep and wakefulness. Our data demonstrate that the lowest irisin concentration promoted a hypoventilation due to a lower $\dot{V}_E$ under room air conditions. In addition, the highest irisin concentration also promoted a reduction in $\dot{V}_E$ and a decreased f_R, predominantly during waking state, suggesting that this myokin acting on central nervous system (CNS) as a tonic inhibitory effect on breathing control. Regarding the hypercapnic stimulus, the animals treated with the highest concentration of irisin centrally showed a greater hyperventilatory response to CO_2 due to a higher $\dot{V}_E$ and lower $\dot{V}O_2$, specifically during wakefulness.During hypoxia exposure, central treatment with both irisin concentrations did not

promote changes in ventilatory and metabolic variables but blunted the hypoxia-induced hypothermia. As to cardiovascular regulation, irisin acting on CNS exerts an excitatory modulation in HR.

In resting conditions, the highest irisin concentration promoted a reduction in $\dot{V}_E$ due to a drop in f_R and also a trend to decrease $\dot{V}O_2$. Ventilation is adjusted according to metabolic demands and promotes gas exchange between the external environment and the blood, capturing oxygen and releasing carbon dioxide. Carrying out gas exchange, together with the metabolic needs is what defines the values of $\dot{V}_E$, f_R and V_T of an individual (Mortola, 2018). During resting conditions, breathing requires minimal energy expenditure and the breathing pattern must adjust to the mechanical properties of the respiratory system. As a ventilatory strategy, increasing tidal volume is generally energetically more costly than increasing respiratory rate, but more efficient compared to increase in respiratory rate since the volume of air reaching the gas exchange surface is increased as a result of a reduction in airflow ratio of air to dead space (Otis, 1954). Therefore, in our study, it is possible that central treatment with irisin is promoting a more efficient ventilatory response, since during ambient air conditions, animals treated with both concentrations of irisin reduced $\dot{V}_E$, while those who received the microinjection with the highest dose also presented a decrease in f_R. During exposure to hypercapnia, the animals treated with the highest dose increased $\dot{V}_E$ and V_T, thus showing that the treatment with irisin enabled the animals to perform gas exchange in a more efficient manner, reducing energy expenditure, even when CO_2 levels were high.

In our study, no change in hypoxic ventilatory response was observed. It is possible that this lack of responses during exposure to hypoxia is because this myokine acts mainly in regions of the CNS that modulate the chemoreception to CO_2 and not the ventilatory response to hypoxia. In fact, FNDC5/irisin has been found to be expressed robustly not only in skeletal muscle (Boström et al., 2012) but also in various regions of brain tissue (Dunn et al., 2013), however, there is no study showing the presence of irisin receptors in the carotid body.

The regulation of respiratory reflexes are considerably different during sleep/wake (Douglas, 2000; Krieger, 2000). As evidenced, the results of the present study showed differences in the findings between sleep/wake states.

During sleep, the treatment with the highest concentration of irisin promoted a reduction in f_R, as well as in $\dot{V}O_2$ and an increase in $\dot{V}_E/\dot{V}O_2$ at resting conditions, while during exposure to hypoxic conditions, the animals treated with both irisin concentrations did not show the hypothermia regulated by exposure to low levels of O_2. On the other hand, during exposure to hypercapnia, there were not found significant differences between control and irisin-treated animals. Therefore, the findings in the ventilatory parameters presented during the waking state were not maintained during sleep, thus evidencing the importance of evaluating these variables and others in both states.

The first discovery related to irisin was correlated to its possible role in the regulation of thermogenesis (Bostrom et al., 2012). Since then, several studies sought to evaluate its promising effect associated with metabolic functions. The study by Zhang et al. (2015) has already reported that the microinjection of irisin in the 3rd ventricle at a much higher concentration (2.5 μg/μL) than those used in the present study increased the $\dot{V}O_2$, as well as the heat production and locomotor activity. The results of the present study demonstrate that animals centrally treated with the higher irisin concentration (1.66 μg/μL) showed a reduction in $\dot{V}O_2$ during hypercapnic conditions in wakefulness and in room air conditions during sleep. Possibly the differences between the studies may be related to the dose used, administration location, as well as the different exposure conditions during the experiments.

Previous study has demonstrated that central injection of irisin (1, 3 and 10 μM caused an increase in body temperature in rats at 16, 24, and 48 hours of interval after its application (Erden et al., 2016). We did not observe changes in T_B in animals treated with irisin or vehicle in sleep/wake states during room air and hypercapnic conditions. Interestingly, the animals that received i.c.v injection of irisin at both concentrations (0.425 and 1.66 μg/μL) did not show a drop in T_B during exposure to hypoxia regardless sleep/wake state. The regulated drop in body temperature during hypoxia is caused by the inhibition of thermogenic responses and peripheral vasodilation to increase the heat loss with the environment and help to reduce body temperature (Bicego et al., 2002). The fact that animals treated with irisin did not present regulated hypothermia during hypoxia may be due to the action of this myokine in regions of thermal control,

increasing thermogenesis during the stimulus or activating areas responsible for the sympathetic control, stimulating vasoconstriction and conserving heat. In fact, a recent study in zebra fish showed that the sympathetic/beta-adrenergic pathway regulates the effects of irisin in cardiovascular regulation and cardiac gene expression in these animals (Sundarrajan et al., 2021).

Regarding cardiovascular parameters, recent studies have demonstrated that irisin participates in cardiovascular control (Zhang et al., 2015). According to the authors, central irisin increases cardiac output and blood pressure due to activation of neurons in the hypothalamic paraventricular nucleus. On the other hand, another study has reported that the microinjection of irisin into the nucleus ambiguus promotes a significant reduction in HR, resulting bradycardia in animals (Brailoiu et al., 2015). As evidenced in the results of the present study, the central treatment with the lowest concentration of irisin promoted an increase in the HR during all conditions in sleep/wake states. On the other hand, the treatment with the highest dose promoted an increase in the HR of the animals when exposed to hypoxic conditions during sleep/wake states and in hypercapnic conditions only during sleep, with no change in MAP in any of the conditions and treatments. The differential effects of irisin on the function of heart rate may be related to difference in dose, region of microinjection and different animal. As mentioned, Brailou et al. (2015), evaluated the effect of irisin in nucleus ambiguous, a specific region that participates in central parasympathetic cardiac control and they observed bradycardic response. In our study, we performed a more diffused injection that probably reached different CNS areas, including regions that participates in the central sympathetic cardiac control, such as, for example, the rostral ventrolateral medulla region (Dampney et al., 2003a,b; Milner and Pickel, 2003). Therefore, the effect of irisin on heart rate control may depend on the region that this peptide is acting.

In conclusion, our data demonstrate that central treatment with irisin with the two concentrations generated a condition of hypoventilation under room air condition due to a lower $\dot{V}_E$ indicating an inhibitory role of this myokine on breathing control, while the treatment with the highest concentration potentiated the hyperventilatory response to CO_2 promoting an increase in $\dot{V}_E$ and inhibiting the $\dot{V}O_2$ during wakefulness. In addition, this myokine has been shown to be

excitatory for HR and capable of attenuating the drop in body temperature under conditions of low O_2 levels.

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Figure Legends

Fig. 1. Effect of central irisin microinjection (0.425 or 1.66 μg/μL) on ventilation ($\dot{V}_E$), tidal volume (V_T), respiratory frequency (f_R), oxygen consumption ($\dot{V}O_2$), respiratory equivalent ($\dot{V}_E / \dot{V}O_2$) and body temperature (T_B) during resting conditions in wakefulness. Values are expressed as mean ± S.E.M. *Represents significant difference compared with control group. #Represents significant difference between irisin groups.

Fig. 2. Effect of central irisin microinjection (0.425 or 1.66 μg/μL) on ventilation ($\dot{V}_E$), tidal volume (V_T), respiratory frequency (f_R), oxygen consumption ($\dot{V}O_2$), respiratory equivalent ($\dot{V}_E / \dot{V}O_2$) and body temperature (T_B) during hypercapnia (7% CO_2) in wakefulness. Values are expressed as mean ± S.E.M. *Represents significant difference compared with control group. #Represents significant difference between irisin groups.

Fig. 3. Effect of central irisin microinjection (0.425 or 1.66 μg/μL) on ventilation ($\dot{V}_E$), tidal volume (V_T), respiratory frequency (f_R), oxygen consumption ($\dot{V}O_2$), respiratory equivalent ($\dot{V}_E / \dot{V}O_2$) and body temperature (T_B) during hypoxia (10% O_2) in wakefulness. Values are expressed as mean ± S.E.M. *Represents significant difference compared with control group.

Fig. 4. Effect of central irisin microinjection (0.425 or 1.66 μg/μL) on mean arterial pressure (MAP) and heart rate (HR) during waking state at resting conditions (A), hypercapnia (B) and hypoxia (C). Values are expressed as mean ± S.E.M. *Represents significant difference compared with control group within the same exposure condition. #Represents significant difference between irisin groups within the same exposure condition.

Fig. 5. Effect of central irisin microinjection (0.425 or 1.66 μg/μL) on ventilation ($\dot{V}_E$), tidal volume (V_T), respiratory frequency (f_R), oxygen consumption ($\dot{V}O_2$), respiratory equivalent ($\dot{V}_E / \dot{V}O_2$) and body temperature (T_B) during resting conditions in sleep state. Values are expressed as mean ± S.E.M. *Represents significant difference compared with control group. #Represents significant difference between irisin groups.

Fig. 6. Effect of central irisin microinjection (0.425 or 1.66 μg/μL) on ventilation ($\dot{V}_E$), tidal volume (V_T), respiratory frequency (f_R), oxygen consumption ($\dot{V}O_2$), respiratory equivalent ($\dot{V}_E / \dot{V}O_2$) and body temperature (T_B) during hypercapnia (7% CO_2) in sleep state. Values are expressed as mean ± S.E.M.

Fig. 7. Effect of central irisin microinjection (0.425 or 1.66 μg/μL) on ventilation ($\dot{V}_E$), tidal volume (V_T), respiratory frequency (f_R), oxygen consumption ($\dot{V}O_2$), respiratory equivalent ($\dot{V}_E / \dot{V}O_2$) and body temperature (T_B) during hypoxia (10% O_2) in sleep state. Values are expressed as mean ± S.E.M. *Represents significant difference compared with control group.

Fig. 8. Effect of central irisin microinjection (0.425 or 1.66 μg/μL) on mean arterial pressure (MAP) and heart rate (HR) during waking state at resting conditions (A), hypercapnia (B) and hypoxia (C). Values are expressed as mean ± S.E.M. *Represents significant difference compared with control group within the same exposure condition. #Represents significant difference between irisin groups within the same exposure condition.

Table 1. Ventilation ($\dot{V}_E$), tidal volume (V_T), respiratory frequency (f_R), oxygen consumption ($\dot{V}_{O_2}$), respiratory equivalent ($\dot{V}_E / \dot{V}_{O_2}$), mean arterial pressure (MAP), heart rate (HR) and body temperature (T_B) of control and irisin-treated (0.425 or 1.66 μg/μL) animals during resting conditions in wakefulness before central microinjection.

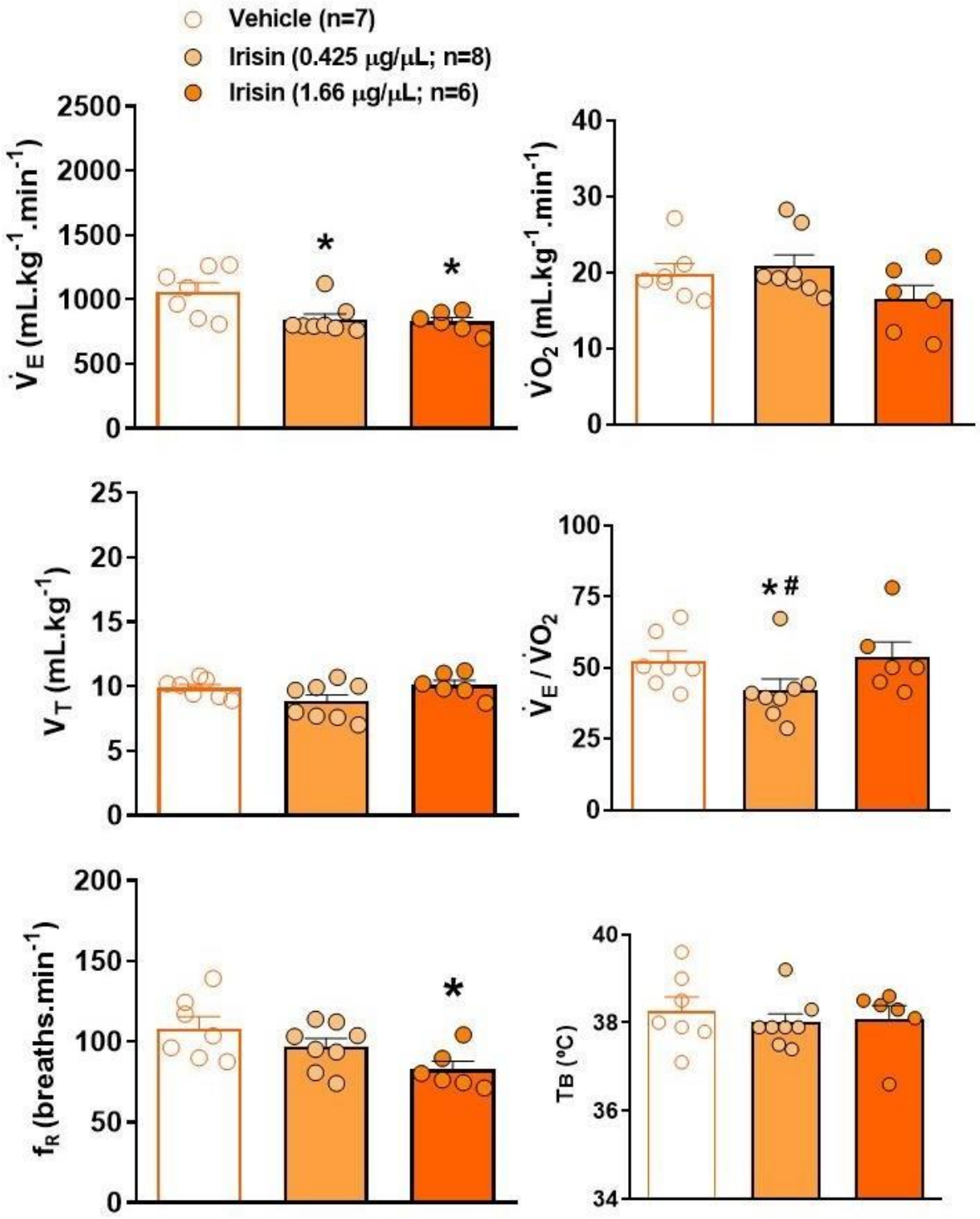
	Groups		
	Vehicle (n=23)	Irisin (0.425 μg/μL; n=23)	Irisin (1.66 μg/μL; n=8)
$\dot{V}_E$ ($mL.Kg^{-1}.min^{-1}$)	857.5 ± 22.1	859.2 ± 29.6	865.7 ± 2.6
V_T ($mL.Kg^{-1}$)	8.9 ± 0.2	8.9 ± 0.2	9.7 ± 0.6
f_R (breaths. min^{-1})	97.2 ± 3.1	96.3 ± 2.4	91.6 ± 6.3
$\dot{V}O_2$ ($mL.Kg^{-1}.min^{-1}$)	19.1 ± 0.7	18.6 ± 0.4	16.6 ± 1.3
$\dot{V}_E/\dot{V}O_2$	46.9 ± 2,3	47.3 ± 2.0	57.3 ± 6.3
MAP (mmHg)	138.7 ± 1.9	135.9 ± 1.5	137.9 ± 3.8
HR (beats min^{-1})	343.5 ± 8.5	331.9 ± 5.2	338.3 ± 12.3
T_B (ºC)	37.9 ± 0.1	38.0 ± 0.1	37.7 ± 0.2

Table 2. Values of arterial pH (pHa), partial pressure of arterial carbon dioxide ($PaCO_2$), partial pressure of arterial oxygen (PaO_2) and plasma bicarbonate (HCO_3^-) after 15 min of microinjection of control and irisin treated animals (0.425 or 1.66 µg/µL) during room air, hypercapnia and hypoxia.

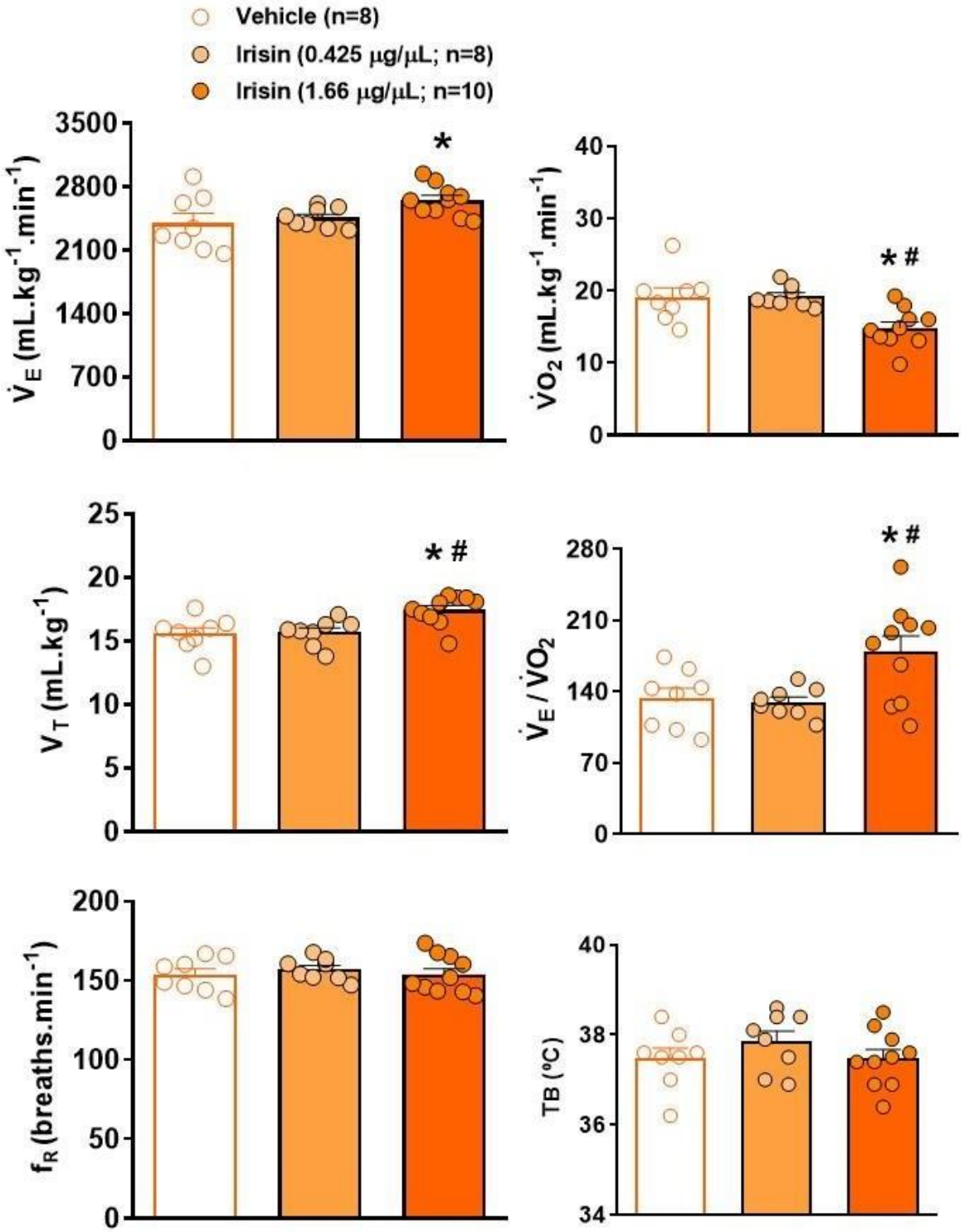
	Vehicle			Irisin (0.425 µg/µL)			Irisin (1.66 µg/µL)		
	Room air (n=7)	Hypercapnia (n=8)	Hypoxia (n=7)	Room air (n=7)	Hypercapnia (n=7)	Hypoxia (n=7)	Room air (n=7)	Hypercapnia (n=7)	Hypoxia (n=5)
pHa	7.42 ± 0.01	7.33 ± 0.01$^{\#+}$	7.60 ± 0.01$^{\#}$	7.44 ± 0.01	7.32 ± 0.01$^{\#+}$	7.61 ± 0.01$^{\#}$	7.46 ± 0.01*	7.30 ± 0.01$^{\#+}$	7.60 ± 0.01$^{\#}$
$PaCO_2$ (mmHg)	36.1 ± 1.8	52.2 ± 1.9$^{\#+}$	19.7 ± 1.2$^{\#}$	32.0 ± 1.0	47.7 ± 2.5$^{\#+}$	19.4 ± 1.2$^{\#}$	28.0 ± 1.6	44.2 ± 3.2$^{\#+}$*	18.8 ± 1.6$^{\#}$
PaO_2 (mmHg)	89.8 ± 3.4	118.6 ± 2.2$^{\#+}$	31.0 ± 1.2$^{\#}$	87.8 ± 2.2	123.6 ± 2.5$^{\#+}$	31.8 ± 1.4$^{\#}$	88.3 ± 1.7	117.1 ± 2.2$^{\#+}$	30.4 ± 1.7$^{\#}$
HCO_3^-	23.2 ± 1.5	27.3 ± 1.0^{+}	19.4 ± 1.1	21.4 ± 0.5	24.5 ± 1.5^{+}	19.5 ± 1.3	19.6 ± 1.0	22.2 ± 1.8*	18.3 ± 1.5

*Indicates significant difference compared with control group within the same exposure condition. $^{\#}$Indicates significant difference compared with room air conditions within the same group. $^{+}$Indicates significant difference between hypercapnia and hypoxia within the same group.

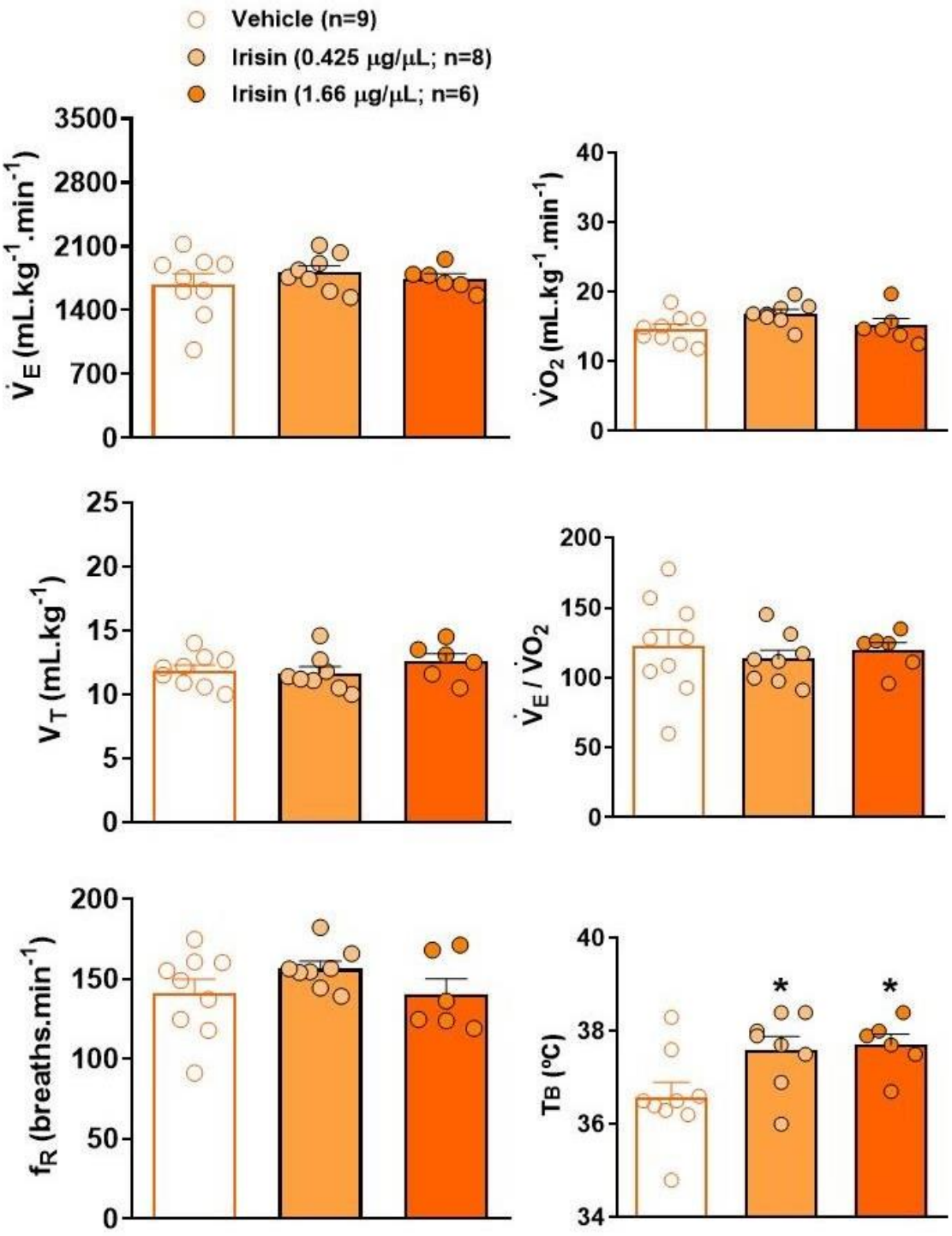
Room air
wakefulness



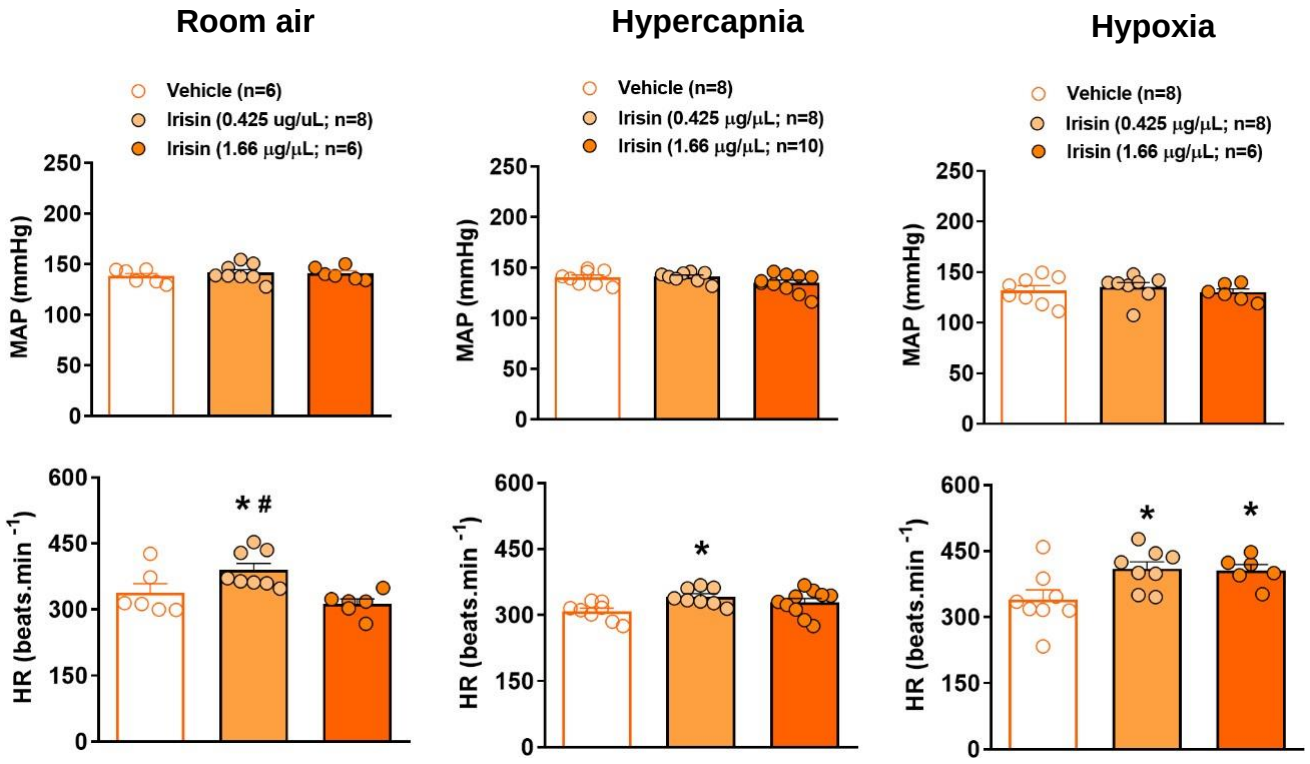
Hypercapnia
wakefulness



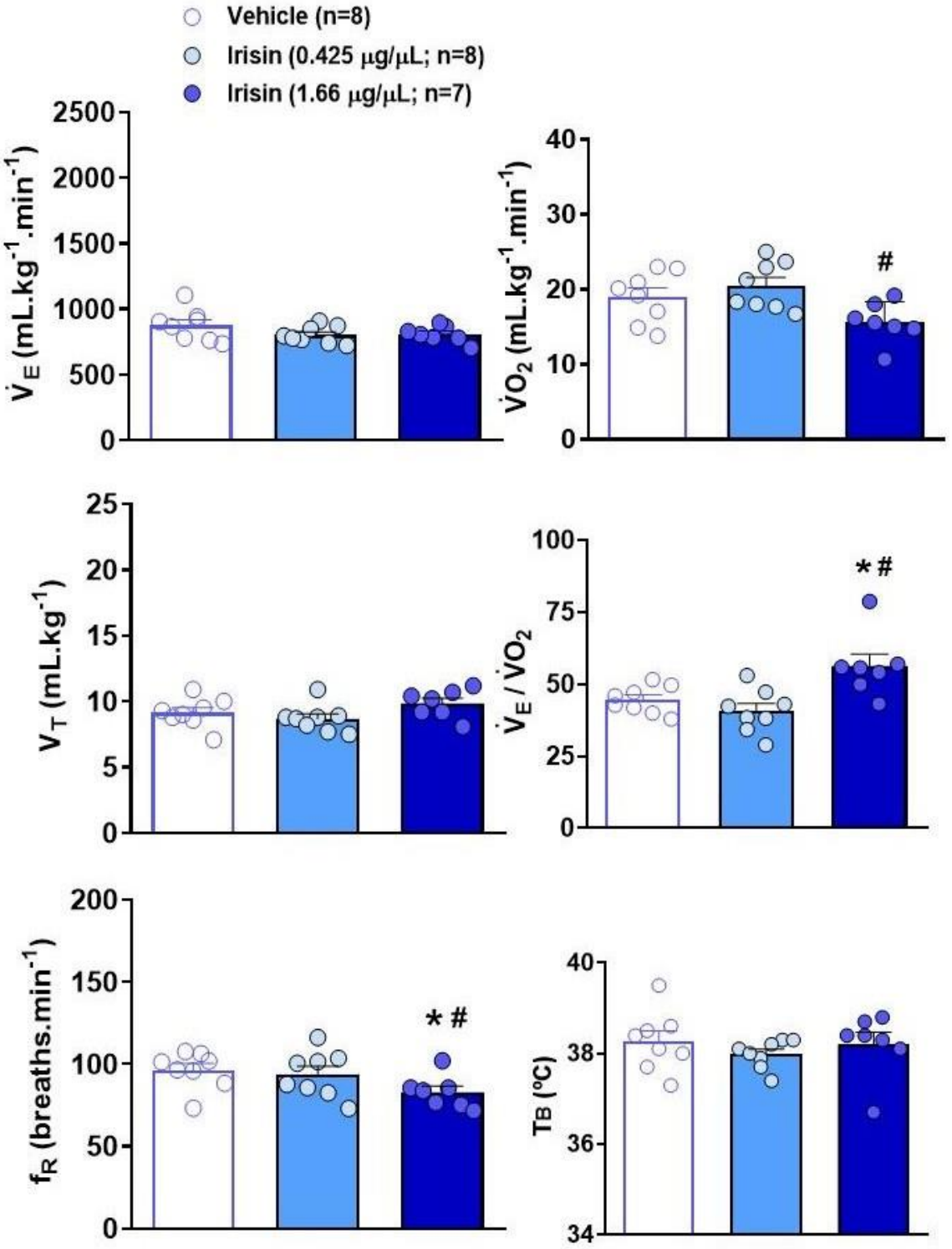
Hypoxia
wakefulness



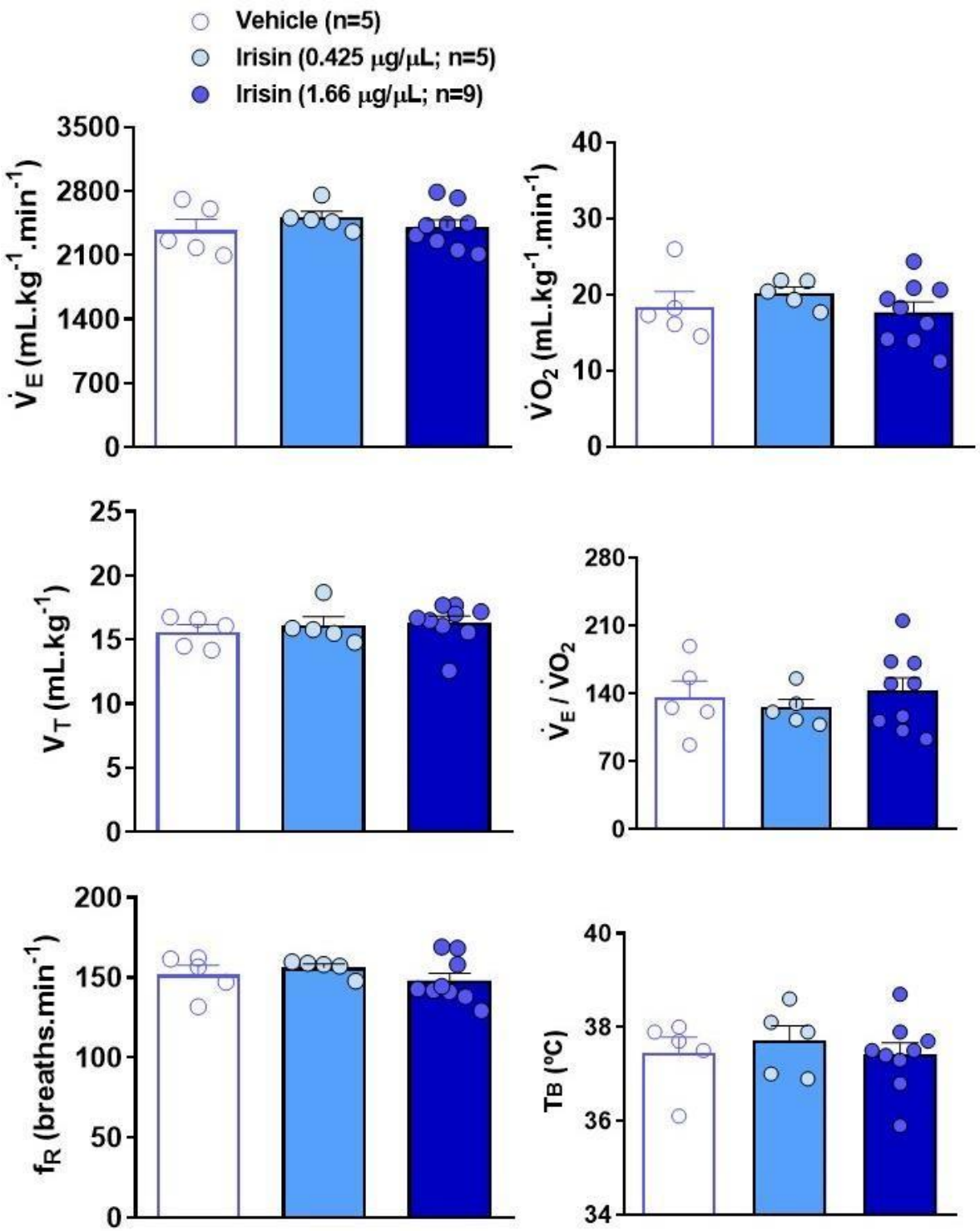
Wakefulness



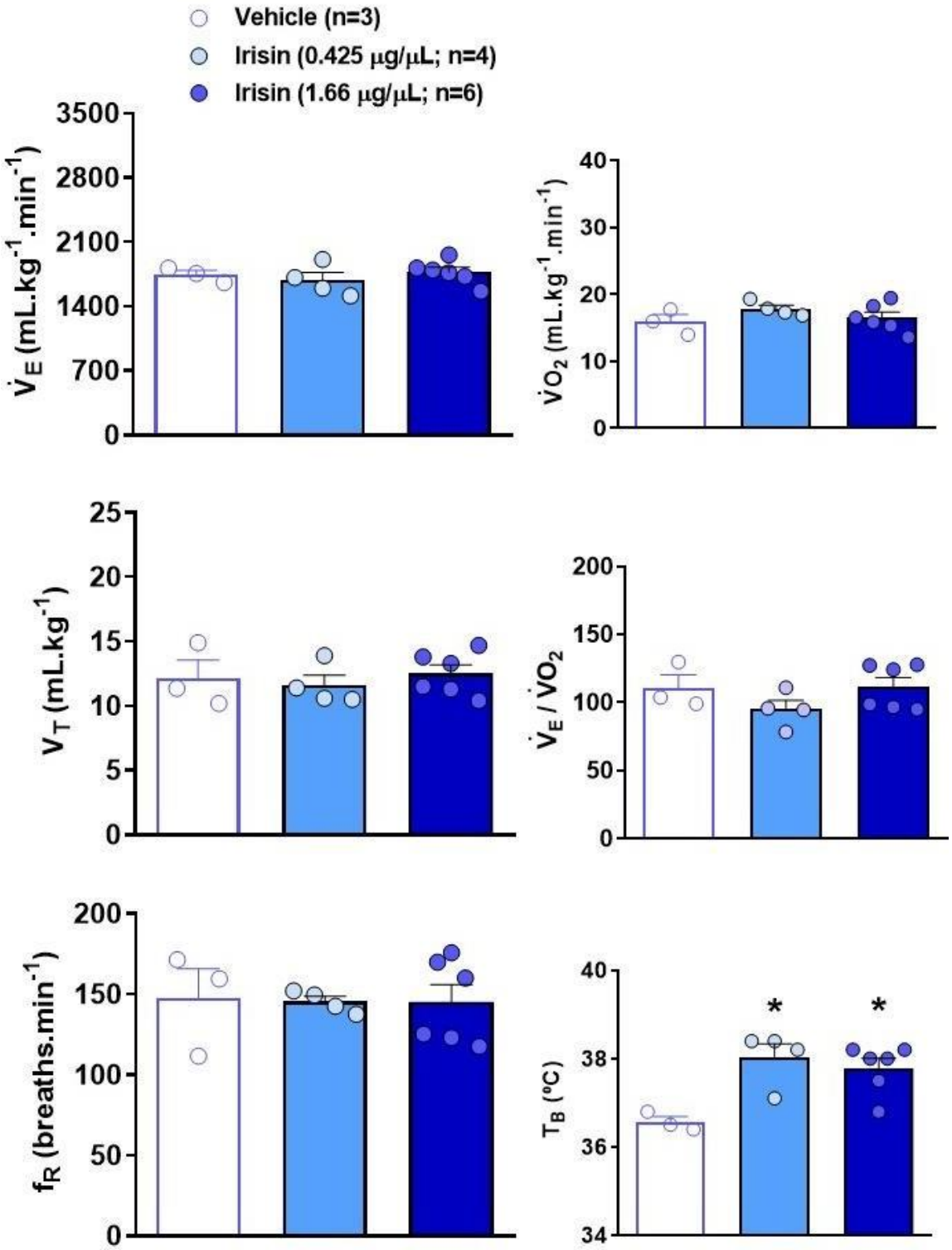
Room air
sleep

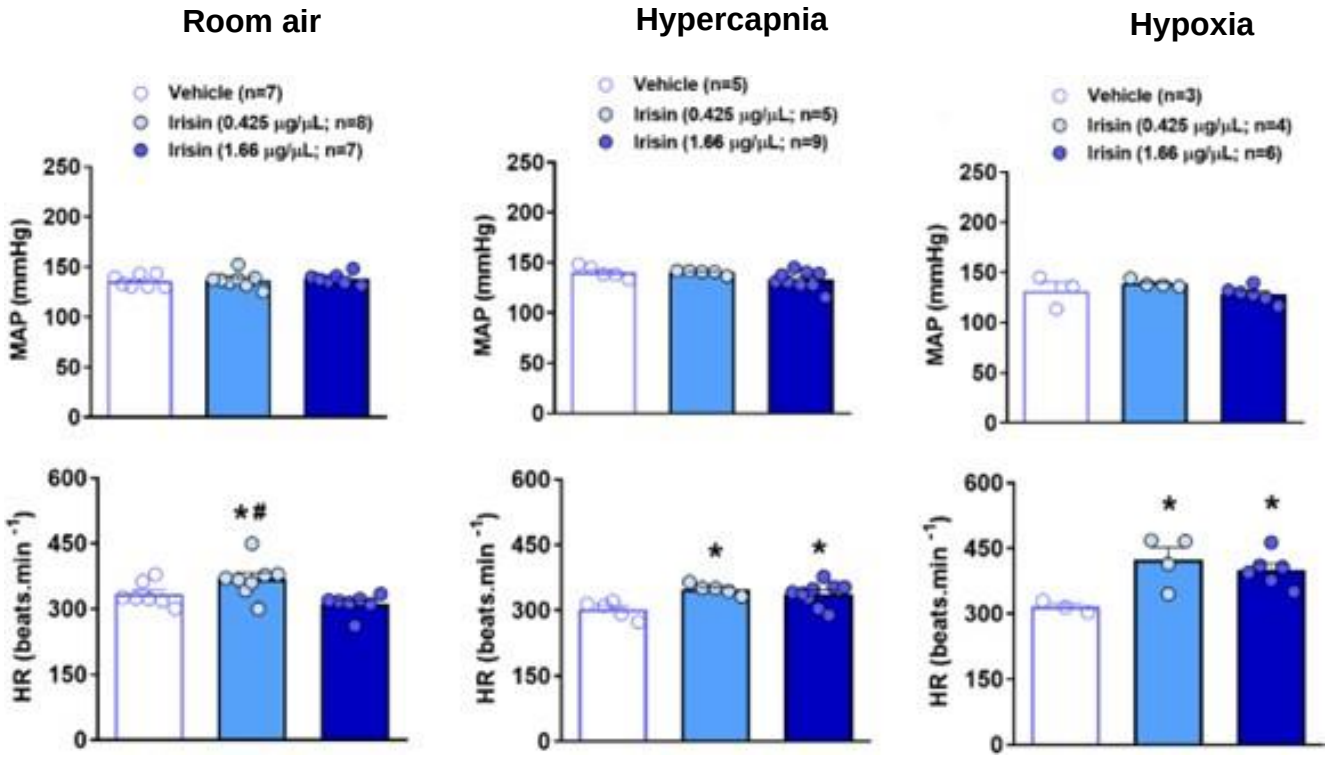


Hypercapnia
sleep



Hypoxia
sleep




Room air
Hypercapnia
Hypoxia
Vehicle (n=7)
Irisin (0.425 μg/μL; n=8)
Irisin (1.66 μg/μL; n=7)
Vehicle (n=5)
Irisin (0.425 μg/μL; n=5)
Irisin (1.66 μg/μL; n=9)
Vehicle (n=3)
Irisin (0.425 μg/μL; n=4)
Irisin (1.66 μg/μL; n=6)
MAP (mmHg)
HR (beats.min -1)
0
50
100
150
200
250
300
450
600
*#
*