UNIVERSIDADE FEDERAL DE SERGIPE
CENTRO DE CIÊNCIAS BIOLÓGICAS E DA SAÚDE
DEPARTAMENTO DE MEDICINA

Avaliação das proteínas envolvidas nas etapas iniciais da via de sinalização da insulina em músculo masseter de animais tratados com dexametasona

Igor Rabelo de França

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Aprovada em:_____de _____de _____

BANCA EXAMINADORA

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"A menos que modifiquemos a nossa maneira
de pensar, não seremos capazes de resolver os problemas
causados pela forma como nos acostumamos a ver o mundo."
(Albert Einstein)

RESUMO

A resistência à insulina (InsR) é causada por alterações moleculares na quantidade e/ou no grau de fosforilação de proteínas a jusante a sinalização das vias do receptor para insulina/receptor do fator de crescimento semelhante a insulina (IR/IGF1R) observados no músculo esquelético, fígado e tecido adiposo. Todavia, existem poucos estudos na via intracelular da insulina no músculo masseter em condições metabólicas alteradas como a obesidade e o diabetes. Esse estudo objetiva analisar a sinalização IR/IGF1R no músculo masseter de ratos tratados com dexametasona (glicocorticoide que em altas concentrações induz manifestações de InsR). Ratos Wistar machos foram divididos em 2 grupos: Controle – injeção intraperitonial de solução salina 0,9%; e Dexametasona – injeção intraperitoneal de solução de dexametasona na razão de 1mg/kg de peso corporal de animal por 10 dias consecutivos. Fragmentos do músculo masseter foram retirados nos tempos zero e após infusão de insulina regular na veia porta para quantificação do peso corporal e peso do músculo masseter e das proteínas IR, PI3K, IRS1, AKT e ERK1. A administração de dexametasona foi capaz de reduzir o peso corporal sem alterar o peso do músculo masseter e a expressão proteica do IR e da PI3K totais, permanecendo inalteradas as quantidades totais de IRS1, AKT e ERK1, quando comparado ao grupo controle. O grau de fosforilação/atividade do IRS1 após estímulo com insulina foi aumentado apenas no grupo controle, enquanto que no grupo tratado, foi ausente. A AKT obteve um aumento do grau de fosforilação em ambos os grupos, porém, no grupo tratado com dexametasona esse aumento foi atenuado. Podemos sugerir que músculo masseter parece possuir grau de fosforilação/atividade distintas de outros territórios musculares.

Palavras-chave: músculo masseter; dexametasona; receptor de insulina.

ABSTRACT

Insulin resistance is partly caused by molecular changes in the level and/or degree of phosphorylation of proteins located downstream of the insulin receptor/insulin-like growth factor receptor (IR/IGF1R) signaling pathway that occurs in the skeletal muscle, liver, and adipose tissue. However, few studies have investigated the intracellular insulin pathway in the masseter muscle under altered metabolic conditions such as obesity and diabetes. Therefore, this study aimed to analyze the IR/IGF1R signaling pathway in the masseter muscle of rats treated with dexamethasone (a glucocorticoid that induces manifestations of insulin resistance at high concentrations). Male Wistar rats were divided into two groups: control group, intraperitoneally injected with 0.9% NaCl (saline solution), and dexamethasone group, intraperitoneally injected with a dexamethasone solution (1 mg/kg body weight) for 10 consecutive days. Sections of the masseter muscle were removed at time zero and after the infusion of regular insulin into the portal vein. The administration of dexamethasone induced body weight loss without changing the masseter muscle weight. In addition, it reduced the expression of total IR and PI3K proteins, with the total levels of IRS1, AKT, and ERK1 remaining unchanged compared with the levels in the control group. The degree of phosphorylation/activity of IRS1 after insulin stimulus increased in the control group but not in the dexamethasone group. The degree of phosphorylation of AKT increased in both groups, but this increase was attenuated in the dexamethasone group. We suggest that the degree of phosphorylation/activity in the masseter muscle is different from that in other muscle territories.

Keywords: masseter muscle; dexamethasone; insulin receptor

LISTA ABREVIATURAS E SIGLAS

ACTH	Hormônio adrenocorticotrófico
CRF	Fator liberador da corticotropina
DM2	Diabetes Mellitus tipo 2
GC	Glicocorticoide
GLUT	Transportadores de glicose (*Glucose Transporter*)
HAS	Hipertensão arterial sistêmica
HDLc	Lipoproteína de alta densidade
I DBSM	I Diretriz Brasileira de Síndrome Metabólica
IGF-1	Fator de crescimento semelhante à insulina tipo 1
IGF1R	(*Insulin-like Growth Factor Type 1 Receptor*)
IMC	Índice de Massa Corporal
IR	Receptor para insulina (*Insulin Receptor*)
IRS	Substrato de recepetores para insulina (*Insulin Receptor Substrates*)
LDLc	Lipoproteína de baixa densidade
NCEP-ATP III	*National Cholesterol Education Program`s Adult Treatment Panel III*
OMS	Organização Mundial de Saúde
PI3K	Fosfatidilinositol-3-cinase (*Phosphatidylinositol-3-kinase*)
PIP3	Fosfatidilinositol-3.4.5-trifosfato
PKB ou AKT	Proteína-cinase B (*Protein Kinase B*)
InsR	Resistência à insulina (*InsR – Insulin Resistance*)
SM	Síndrome metabólica

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I-REVISÃO DE LITERATURA

1.1 Insulina

A insulina é um hormônio anabólico com ampla ação metabólica envolvido na regulação da glicose e, consequentemente, dos carboidratos, lipídios e proteínas. Sua síntese, armazenamento e secreção dá-se no pâncreas, mas especificamente pelas células beta-pancreáticas nas ilhotas de Langerhans (HOJLUND, 2014). A liberação de insulina é feita de forma pulsátil, bifásica e dose-dependente dos níveis de glicose, sendo um pico precoce e rápido acontecendo cinco a seis minutos após estimulação (fase da insulina pré sintetizada e armazenada) e outro mais prolongado e gradual levando cerca de 60 minutos para ser realizado (fase da insulina recém-sintetizada) (KOMATSU *et al.,* 2013). Essa secreção é influenciada por diversos fatores agonistas e antagonistas combinados os quais mantêm os níveis de glicose circulante em um intervalo fisiológico aceitável. Por essa razão, a glicose se mostra como o principal fator estimulador e de amplificação da ativação das células beta-pancreáticas e da liberação de insulina. Outros mecanismos também influenciam nessa ativação/liberação como fatores nutricionais (aminoácidos, ácidos graxos livres, cetonas), hormonais (peptídio semelhante ao glucagon 1, glucagon, gastrina, secretina, peptídeo vasoativo intestinal) e neurológicos (acetilcolina e noradrenalina), sendo um sistema complexo, dinâmico e repleto de interações, inclusive ambientais (KOMATSU *et al.,* 2013; MARCHETTI *et al.,* 2017).

O receptor para insulina (IR) é composto de duas subunidades α e β que agem de maneira alostérica permitindo a inibição da subunidade β pela subunidade α. O IR apresenta uma concentração elevada principalmente na musculatura esquelética, fígado e tecido adiposo. Dessa forma, promove a captação de glicose no músculo esquelético e tecido adiposo, além de estimular a síntese de glicogênio pelo músculo esquelético e de suprimir a gliconeogênese hepática e a lipólise nos adipócitos. (CAMPOREZ *et al.,* 2013). A insulina exerce sua função através de uma rede proteica iniciada a partir da sua interação com o IR de tirosina cinases transmembrana na célula. Estes eventos cursam com a desinibição da subunidade β que é capaz de se transfosforilar e ativar proteínas a jusantes da via de sinalização do receptor para insulina/fator de crescimento semelhante à insulina (IR/IGF1) destacando-se os substratos de receptores para insulina (IRS). Esses substratos são fosforilados em resíduos de tirosina e atuam em diversas vias metabólicas para mediar suas ações biológicas, principalmente as vias que regulam o metabolismo intermediário e a que desempenham um papel no controle de processos de crescimento e mitoses. (DEFRONZO *et al.*, 2015; ZHOU *et al.*, 2017).

Os IRSs apresentam funções distintas, porém, podem ter respostas compensatórias entre si. As isoformas do substrato 1 (IRS1) e do substrato 2 (IRS2) parecem possuir ação central no balanço glicêmico corporal. O IRS1 está envolvido nas ações principais da insulina no músculo esquelético: captação de glicose e armazenamento de glicogênio. Em relação ao IRS2, por predominar no fígado, é responsável pelas ações periféricas nesse tecido e, nas ilhotas pancreáticas, está envolvido na proliferação de células beta pancreáticas. Essas observações foram possíveis devido a experimentos que envolvem a deleção total (knockout) do IRS1 em ratos exibiram retardo no crescimento, resistência à insulina, porém sem diabetes (hiperglicemia) e aqueles knockout do IRS2 que manifestaram deficiência à insulina para diabetes e hipoplasia das células pancreáticas. Os outros substratos apresentam efeitos menos importantes nessa via (PREVIS *et al.,* 2000; THIRONE *et al.*, 2006).

A partir da fosforilação do IRS1 e do IRS2 ocorre a ativação de diversas cinases subsequente, dentre elas, a fosfatidilinositol-3-cinase (PI3K). Existem duas subunidades diferentes na PI3K: uma catalítica (p110) e outra regulatória (p85). A ligação dos IRSs na subunidade p85 dissocia-se da subunidade p110 promovendo a catalização de fosfatidilinositol-4,5-bifosfato em fosfatidilinositol-3,4,5-trifosfato (PIP3), como consequência (HOJLUND, 2014; ZHOU *et al.*, 2017), a proteína-cinase B (PKB ou Akt), uma serina/treonina é ativada. Esta molécula, que possui três isoformas (Akt1, Akt2, Akt3), é translocada para membrana plasmática quando é fosforilado seus resíduos de serina 473 e treonina 308, exercendo importante papel no transporte de glicose-dependente de insulina, síntese de proteínas, lipídios e glicogênio, controle da gliconeogênese e diferenciação celular (CAMPOREZ *et al.,* 2013; ZHOU *et al.,* 2017).

A captação de glicose na célula é realizada por difusão facilitada com ajuda de proteínas transportadoras devido a impermeabilidade da membrana plasmática à glicose. A família de transportadores de glicose (GLUT) é composta por várias isoformas e cada uma apresenta distribuição diferente nos diversos tecidos. No músculo esquelético e no tecido adiposo, predomina o subtipo 4 (GLUT4), responsável pela captação de glicose estimulada pela insulina (KHAN; PESSIN, 2002; ESTEVES *et al.,* 2017). O GLUT4, em baixas concentrações de insulina, predomina a sua localização no espaço intracelular (mais de 90%) dentro de vesículas de armazenamento. Após estimulação com insulina, uma das vias de sinalização da insulina (IR/IRS/PI3K/Akt) transloca o GLUT4 das vesículas de armazenamento para a membrana celular para exercer sua função (BOGAN, 2012; ESTEVES *et al.,* 2017). Em estudos knockout GLUT4 realizados com ratos induziu redução na captação de glicose,

hiperinsulinemina e resistência periférica à insulina (RI), comprovando o papel indispensável dessa proteína no metabolismo da glicose (ZISMAN *et al.,* 2000; ABEL *et al.,* 2001).

1.2 Resistência à insulina

A RI é caracterizada como uma condição clínica desenvolvida por uma resposta diminuída à ação da insulina nos órgãos alvo por alterações nas vias de captação da glicose, o que resulta em altos níveis sanguíneos desse carboidrato. De maneira compensatória, são necessárias altas concentrações de insulina, superiores ao normal, para manter a glicemia em níveis fisiológicos aceitáveis e sem comprometimento das funções metabólicas. Com o tempo, a necessidade cada vez maior de insulina causando modificações em diversos compartimentos da ultraestrutura intracelular alterando gradativamente o limiar de liberação de insulina, a perda da funcionalidade celular e, por fim, a falência completa do pâncreas (GUO, 2013; DEFRONZO *et al.,* 2015; MARCHETTI *et al.,* 2017).

Alguns autores demonstraram que a liberação de insulina estimulada pela glicose em pacientes com RI era menor, o que podem contribuir para a gênese da RI (DEL GUERRA *et al.,* 2005). Na maioria dos casos, a RI é associada às manifestações em defeitos localizados após o receptor para insulina a nível celular por modificações moleculares na quantidade e/ou no grau de fosforilação de proteínas envolvidas na cascata de sinalização do IR/IGF1 interferindo em múltiplos aspectos metabólicos e diferentes aspectos da função celular. Na musculatura esquelética, diminuição da sinalização do IR/IGF1, contribui para o estado hiperglicêmico constatado na RI. Entretanto, a real origem dessa desordem ainda continua pouco esclarecida. As principais hipóteses envolvidas incluem: regulação negativa, deficiência ou polimorfismo genético da fosforilação da tirosina do IR, IRSs ou PI3K, ou envolvendo anormalidades na função da proteína transportadora de glicose quatro (GLUT 4) (SAINI, 2010; GUO, 2013; DEFRONZO *et al.,* 2015).

A RI apresenta uma forte correlação com a fisiopatologia de diversas doenças que desde 1980 vem sendo caracterizada como síndrome metabólica (SM) ou síndrome da resistência à insulina ou síndrome “X” (GUO, 2013). A Organização Mundial da Saúde (OMS) preconiza como critério central para avaliação do indivíduo portador de SM o desenvolvimento da RI. A SM é definida como um conjunto variável de fatores de riscos metabólicos e fatores inerentes ao indivíduo correlacionados que contribuem diretamente para a morbi-mortalidade da população (GRUNDY, 2005). O significado da SM surge a partir das transformações que essa síndrome prediz em várias doenças futuras como o Diabetes Mellitus tipo 2 (DM2),

hipertensão arterial sistêmica (HAS), dislipidemia, hipertrigliceridemia e obesidade. Além disso, várias outras condições são citadas com forte associação com a SM como síndrome do ovário policístico, estados pró-inflamatórios, estados pró-trombóticos, doença hepática gordurosa não alcoólica, microalbuminúria, hiperuricemia, *acanthosis nigricans* e disfunção endotelial sendo necessárias investigações diagnósticas no paciente que desenvolve essa síndrome mesmo não fazendo parte dos critérios clássicos (GRUNDY, 2005; MBATA *et al.*, 2017).

Existem diversos critérios clínicos e laboratoriais utilizados para a definição da SM. Contudo, o mais amplamente utilizado e também aceito pela I Diretriz Brasileira para Síndrome Metabólica (I-DBSM) pela sua facilidade e aplicabilidade clínica é a definição da *National Cholesterol Education Program`s Adult Treatment Panel III* (NCEP-ATP III) que engloba os itens da tabela abaixo (Tabela 1), sendo necessários pelo menos três dos critérios para confirmação:

Tabela 1

Tabela 1: Critério diagnóstico da SM, segundo a NCEP-ATP III	
Presença de pelo menos três dos fatores de risco seguinte:	
Glicemia em jejum	≥ 100 mg/dL
Triglicerídes	≥ 150 mg/dL
HDL-colesterol	Homem < 40 mg/dL Mulher < 50mg/dL
Obesidade central	CA Homem > 102 cm CA Mulher > 88 cm
Hipertensão	Pressão sistólica ≥ 130 mmHg ou Pressão diastólica ≥ 85 mmHg

Fonte: Adpatado da NCEP-ATP III

*CA - Circunferência abdominal

Nesse contexto, a mudança no perfil metabólico da população mundial somado a condições genéticas predisponentes assumem um papel central na gênese dessas alterações. A exposição a diversos fatores obesogênicos presentes no estilo de vida atual dentre eles o sedentarismo, a facilidade no acesso a alimentos gordurosos e hipercalóricos e a baixa ingesta de fibras aumentam a incidência e a prevalência de pessoas com sobrepeso ou obesas e suas

consequências (KELLY *et al.,* 2008; HOUMARD *et al.,* 2012; CAPEHORN *et al.,* 2016). Em estudo realizado por Pan *et al.* (2015) relacionou um maior risco de desenvolver RI na população idosas, obesas e sedentárias e que os índices de adiposidades (índice de massa corpórea – IMC, circunferência abdominal, relação cintura-altura e relação cintura-quadril) foram relacionados com o RI independente do gênero da população analisada. Esse quadro pode contribuir para o desenvolvimento do diabetes.

O paciente pré-diabético e/ou com DM2 também foi demonstrado relevância dentro da SM pela sua associação com a RI e com a obesidade e por ser a endocrinopatia mais comum nesses indivíduos, sendo considerado um dos grandes desafios da saúde do século (GUH *et al.,* 2009; SAINI, 2010; GLAUBER, 2013; SANDOUK; LANSANG, 2017). Apesar de ter ocorrido um melhor controle glicêmico pela população entre os anos 2007-2010, quando comparado com aqueles em 1999-2002, ainda existe em torno de 33-44% de pacientes que não atingiram a meta aceitável (glicemia de jejum < 100 mg/dL) fazendo com que essa comorbidade, junto com outras três doenças crônicas não transmissíveis constassem na Declaração Política sobre Prevenção e Controle das Doenças Não-Transmissíveis de 2011 com objetivos gerais de prevenção e controle para serem alcançados até 2025 (OMS, 2016; DIABETES CARE, 2017).

No DM2 estão envolvidas alterações progressivas na ação da insulina ou RI, redução na secreção do hormônio pelo pâncreas ou um defeito em ambos os processos. Na fase assintomática da doença, a RI e a disfunção pancreáticas já estão presentes assim como um estado inflamatório e pró-trombótico que expõem a vasculatura a um ambiente aterotrombótico o que aumenta o risco de complicações agudas e crônicas macro e microvasculares destacando as crises hiperglicêmicas (cetoacidose diabética e estado hiperglicêmico hiperosmolar), hipoglicemia (tratamentos inadequados), retinopatia diabética, doença renal do diabetes, neuropatia diabética e pé diabético (SAINI, 2010; MERCURIO *et al.,* 2012; GUO, 2013 DEFRONZO *et al.,* 2015).

1.3 Glicocorticóide

Os glicocorticoides (GC) são uma classe de hormônios esteroides lipossolúveis secretados pela região cortical das glândulas suprarrenais (também chamada de adrenal), mais precisamente, na zona fasciculada (em maior quantidade) e na zona reticulada (em menor quantidade) capaz de atravessar a membrana devido à sua estrutura hidrofóbica e interagir nos receptores específicos no citoplasma, exercendo atividades no metabolismo energético e dos lipídios e na resposta adaptativa a situações de estresse (STREHL; BUTTGEREIT, 2013). No

corpo humano, são representados pelo cortisol e pela corticosterona que são formados, assim como os outros hormônios esteroides, a partir do colesterol, principalmente, sob a forma de lipoproteína de baixa densidade (LDL-colesterol) proveniente da circulação (STREHL; BUTTGEREIT, 2013). Os GC são secretados a partir da ativação do eixo hipotálamo-hipófise-adrenal pelo hormônio adrenocorticotrófico (ACTH). O controle desse eixo é realizado por mecanismos de feedback positivos, quando a dosagem desses hormônios está baixa, e negativos, quando os níveis na circulação desses hormônios estão elevados provendo uma resposta apropriada em face aos mais diversos estímulos (NICOLAIDES *et al.,* 2014). Em resumo, o hipotálamo é estimulado a liberar o fator liberador da corticotropina (CRF) que, por sua vez, resulta na síntese e secreção do ACTH pelo lobo anterior da hipófise que age no córtex da adrenal produzindo o GC (NICOLAIDES *et al.,* 2014; KOCH *et al.,* 2017).

A liberação dos GCs é feita de forma pulsátil em intervalos aproximados de uma hora com amplitudes que variam durante o dia no qual atinge seu pico máximo sanguíneo às nove horas da manhã e alcança suas concentrações mais baixas a meia-noite obedecendo o sistema circadiano influenciando, também, outros tecidos periféricos (KOCH *et al.,* 2017. Seus efeitos pleiotrópicos são mediados pelos receptores pertencentes à superfamília de receptores nucleares de glicocorticoide (receptores humanos de mineralocorticoide e glicocorticoide) com padrões de expressão ampla em quase todo o corpo (NICOLAIDES *et al.,* 2014).

Dentre as diversas funções desse esteroide podemos citar a influência nos transcriptomas de leucócitos humanos, a regulação do metabolismo oxidativo no fígado, músculo e tecido adiposo, coordenação dos níveis pressóricos e tônus muscular no sistema cardiovascular, influenciam em numerosas funções no sistema nervoso central e, principalmente, está envolvido em vários componentes que regulam a quantidade e a qualidade das respostas imunológicas/inflamatórias (NICOLAIDES *et al.,* 2014; VAN RAALTE; DIAMANT, 2014). Por essa razão, desde a década de 1940, os GCs sintéticos vem sendo utilizados amplamente na prática clínica diária como potente anti-inflamatório e imunossupressor dose dependente em diversas condições inflamatórias: doenças reumáticas (artrite reumatoide), doenças renais (vasculites/glomerulonefrites), doenças onco-hematológicas (linfoma, mieloma múltiplo), doenças infecciosas (meningites), doenças pulmonares (doença pulmonar obstrutiva crônica), gastroenterologia (hepatites autoimunes), medicina emergencista (reações alérgicas). Entretanto, a manutenção da terapêutica é limitada em virtude dos efeitos adversos que essa droga pode ocasionar diferindo de acordo com o tempo

de tratamento e a dosagem utilizada (STREHL; BUTTGEREIT, 2013; VAN RAALTE; DIAMANT, 2014; VAN DER GOES *et al.*, 2016).

O excesso de GCs endógeno causado pelo hipercortisolismo ou exógeno por administração de fármacos pode levar a uma série de patologias que envolvem osteoporose por aumento na reabsorção e redução da formação óssea, glaucoma, retenção de líquidos, aumento da susceptibilidade a infeções por imunossuprimir o paciente, úlceras gástricas, doenças psiquiátricas, atrofia e desgaste de pele e de músculo pelo comprometimento nas vias de sinalização de síntese e degradação proteica, aumento da taxa de lipólise, aumento da gordura localizada, diminuição na captação de glicose. Além disso, o uso crônico é capaz de contribuir para quadro semelhante à síndrome de Cushing e para o desenvolvimento de RI e suas consequências (DM2, obesidade central, HAS, dislipidemia, esteatose hepática, etc) (VAN RAALTE; DIAMANT, 2014; HANSEN *et al.*, 2014; VAN DER GOES *et al.*, 2016).

Os GCs são o grupo de drogas mais associados a efeitos diabetogênicos, ele pode induzir hiperglicemia em pacientes sem histórico prévio de diabetes mellitus ou agravar o controle glicêmico em pacientes diabéticos. Além do tempo e da dose utilizada, a idade avançada, histórico familiar de diabetes, IMC elevados atuam como fatores de risco para essa condição (SUH; PARK, 2017). Apesar das informações acerca da prevalência e da incidência da hiperglicemia induzida por GCs serem limitadas, sabe-se que o diagnóstico e o tratamento são bastante subestimados pelos profissionais de saúde pela falta de um critério de diagnóstico definido e de linhas de orientação. Todavia, preconiza-se, de forma semelhante ao DM2, o rastreio precoce em todos os pacientes tratados com doses médias/altas de GC, modificações dos fatores de risco e, quando necessário, uso de hipoglicemiantes orais e insulina exógena (BRADY *et al.*, 2014; PAREDES; ALVES, 2016; SUH; PARK, 2017).

A regulação metabólica é também alterada pela ação do GCs e da RI nos tecidos periféricos (fígado, músculo e tecido adiposo). Em estudos experimentais, a dexametasona, um potente análogo sintético com 25 a 30 vezes mais forte que o cortisol, é uma das drogas mais utilizadas para induzir um quadro de resistência à ação da insulina em ratos (PASIEKA; RAFACHO, 2016). Em outro estudo proposto por Barel *et al.* (2009), realizou-se tratamento com dexametasona 1mg/kg de peso corporal em modelo experimental com roedores, esse esteroide sintético foi capaz de induzir redução do peso corporal, bem como atrofia muscular e RI na musculatura esquelética.

1.4 Músculo Masseter

O músculo masseter é um dos quatro músculos da mastigação junto com os músculos temporal, pterigoide lateral e pterigoide medial (FEHRENBAH; HERRING, 2005). Ele exerce função na mastigação, na salivação e na fala por revestir praticamente todo o ramo da mandíbula inserindo-se no processo maxilar do osso zigomático na porção superficial-superior estendendo posteriormente até a porção medial do arco zigomático. Sua localização dá-se nas porções laterais da face e é composta por três diferentes camadas de fibras: superficial, intermediária e profunda. Essa distribuição configura ao músculo uma vasta gama de movimentos sobre a articulação temporomandibular além de permitir uma interação com diversos músculos da face, inclusive com músculos da língua (WIDMER; ENGLISH; MORRIS-WIMAN, 2007).

A complexa inter-relação dos músculos da mastigação e dos ossos da face é observada em alguns experimentos. Em estudos realizados com a remoção e/ou a desnervação do músculo masseter de ratos (CARTER; HARKNESS, 1995; RODRIGUES *et al.,* 2009; MAYNE *et al.*, 2015) promovem alterações no esqueleto facial levando a perda de massa no músculo, hipertrofia do músculo temporal ipsilateral (processo compensatório) e diminuição do ângulo e da incisura mandibular. Assim como todos os músculos da mastigação, o masseter possui grande importância para o desenvolvimento dos acidentes ósseos através de forças biomecânicas diretas (nos pontos de inserção) e indiretas (nos pontos de oclusão). Foi verificado que a fratura de um dos arcos zigomáticos, principal local de inserção superior do masseter, gera deformidades na face de roedores. Além disso, constata-se relação entre perda de funcionalidade de um desses músculos e hipotrofia das estruturas ósseas da face (PROCÓPIO *et al.*, 2002).

Em outros estudos, as fibras do músculo masseter são influenciadas pelo dimorfismo sexual. Em machos, as fibras do músculo masseter estimuladas por respostas hormonais intracelulares decorrentes da ação da testosterona causam uma transformação fenotípica de parte de suas fibras lentas em fibras rápidas. Não se sabe ao certo o significado funcional dessa alteração, porém, foi sugerido que a predominância de fibras rápidas nesse músculo deva-se a um possível aumento na geração de força durante a mastigação (WIDMER; ENGLISH; MORRIS-WIMAN, 2007). Em um experimento (ABE *et al.,* 2008) relacionando a função dos músculos da mastigação e as propriedades de suas fibras evidenciou que o músculo temporal apresenta maiores níveis de contração e maior força, seguido pelo músculo masseter o qual apresentava predominância de fibras rápidas como citado anteriormente. Os músculos pterigoides, por sua vez, apresentaram menores forças mecânicas. Foram constatadas

semelhanças entre humanos e ratos durante a mastigação uma vez, ambos possuem maiores forças nos músculos temporal e masseter e que os pterigoides servem para ajustar a função da mandíbula (ABE *et al.,* 2008).

Análise da via de sinalização da insulina no músculo masseter é escassa, essa musculatura esquelética amplamente recrutada durante o processo mastigatório e fala. Além desta evidência, existem poucos estudos correlacionando o tratamento com GCs sintéticos e sua ação sobre o músculo masseter e sua interação com o IR/IGF1.

Referências

ABE, S. et al. Relationship between Function of Masticatory Muscle in Mouse and Properties of Muscle Fibers. **The Bulletin Of Tokyo Dental College,** [s.l.], v. 49, n. 2, p.53-58, 2008. Tokyo Dental College.

ABEL, E. D. et al. Adipose-selective targeting of the GLUT4 gene impairs insulin action in muscle and liver. **Nature,** [s.l.], v. 409, n. 6821, p.729-733, 8 fev. 2001. Springer Nature.

AMERICAN DIABETES ASSOCIATION. Promoting health and reducing disparities in populations. Sec. 1. In Standards of Medical Care in Diabetes 2017. **Diabetes Care 2017**, v. 40, n. suppl. 1, p.6-10.

BAREL, M. et al. Exercise training prevents hyperinsulinemia, muscular glycogen loss and muscle atrophy induced by dexamethasone treatment. **European Journal Of Applied Physiology,** [s.l.], v. 108, n. 5, p.999-1007, 6 dez. 2009. Springer Nature.

BOGAN, J. S.. Regulation of Glucose Transporter Translocation in Health and Diabetes. **Annual Review Of Biochemistry,** [s.l.], v. 81, n. 1, p.507-532, 7 jul. 2012. Annual Reviews.

BRADY, V. J. et al. Management of Steroid-Induced Hyperglycemia in Hospitalized Patients With Cancer: A Review. **Oncology Nursing Forum,** [s.l.], v. 41, n. 6, p.355-365, 29 out. 2014. Oncology Nursing Society (ONS).

CAMPOREZ, J. P. G.; ALMEIDA, F. N.; MARÇAL, A. C..Efeitos do exercício físico sobre a via de sinalização da insulina. **Revista Mackenzie de Educação Física**, v. 12, n. 2, p.172-186, 2013.

CAPEHORN, M. S.; HASLAM, D. W.; WELBOURN, R.. Obesity Treatment in the UK Health System. **Current Obesity Reports,** [s.l.], v. 5, n. 3, p.320-326, 28 jun. 2016. Springer Nature.

CARTER, G. M.; HARKNESS, E. M.. Alterations to mandibular form following motor denervation of the masseter muscle. An experimental study in the rat. **Journal of Anatomy**, v. 186, n. 3, p.541-548, jun. 1995.

DEFRONZO, R. A. et al. Type 2 diabetes mellitus. **Nature Reviews Disease Primers,** [s.l.], p.15019-15041, 23 jul. 2015. Springer Nature.

DEL GUERRA, S. et al. Functional and molecular defects of pancreatic islets in human type 2 diabetes. **Diabetes**, v. 54, mar. 2005.

ESTEVES, J. V.; ENGUITA, F. J.; MACHADO, U. F.. MicroRNAs-Mediated Regulation of Skeletal Muscle GLUT4 Expression and Translocation in Insulin Resistance. **Journal Of Diabetes Research,** [s.l.], v. 2017, p.1-11, 2017. Hindawi Limited.

FEHRENBACH M. J.; HERRING S. W.. **Anatomia Ilustrada Cabeça e Pescoço, 2° Edição**. Barueri-SP: Editora Manole, 2005. 362p.

GLAUBER, H.. Preventing Type 2 Diabetes Mellitus: A Call for Personalized Intervention. **The Permanente Journal,** [s.l.], v. 17, n. 3, p.74-79, 1 ago. 2013. The Permanente Press.

GRUNDY, S. M.. Diagnosis and Management of the Metabolic Syndrome: An American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. **Circulation,** [s.l.], v. 112, n. 17, p.2735-2752, 25 out. 2005. Ovid Technologies (Wolters Kluwer Health).

GUH, D. P. et al. The incidence of co-morbidities related to obesity and overweight: A systematic review and meta-analysis. **Bmc Public Health,** [s.l.], v. 9, n. 1, p.1-20, 25 mar. 2009. Springer Nature.

GUO, S.. Insulin signaling, resistance, and the metabolic syndrome: insights from mouse models into disease mechanisms. **Journal Of Endocrinology,** [s.l.], v. 220, n. 2, p.1-23, 26 nov. 2013. BioScientifica.

HANSEN, K. E. et al. A systematic review and meta-analysis of glucocorticoid-induced osteoporosis in children. **Seminars In Arthritis And Rheumatism,** [s.l.], v. 44, n. 1, p.47-54, ago. 2014. Elsevier BV.

HOJLUND, K.. Metabolism and insulin signaling in common metabolic disorders and inherited insulin resistance. **Danish medical journal**, v. 61, n. 7, p.B4890-4930, may 2014..

HOUMARD, J. A.; PORIES, W. J.; DOHM, G. L.. Severe Obesity. **Exercise And Sport Sciences Reviews,** [s.l.], v. 40, n. 4, p.204-210, out. 2012. Ovid Technologies (Wolters Kluwer Health).

I DIRETRIZ BRASILEIRA DE DIAGNÓSTICO E TRATAMENTO DA SÍNDROME METABÓLICA. **Arquivos Brasileiros de Cardiologia**, v. 84, n. suple. 1, abr. 2005.

KELLY, T. et al. Global burden of obesity in 2005 and projections to 2030. **International Journal Of Obesity,** [s.l.], v. 32, n. 9, p.1431-1437, 8 jul. 2008. Springer Nature.

KHAN, A.; PESSIN, J.. Insulin regulation of glucose uptake: a complex interplay of intracellular signalling pathways. **Diabetologia,** [s.l.], v. 45, n. 11, p.1475-1483, 1 nov. 2002. Springer Nature.

KOCH, C. E. et al. Interaction between circadian rhythms and stress. **Neurobiology Of Stress,** [s.l.], v. 6, p.57-67, fev. 2017. Elsevier BV.

KOMATSU, M. et al. Glucose-stimulated insulin secretion: A newer perspective. **Journal Of Diabetes Investigation,** [s.l.], v. 4, n. 6, p.511-516, 15 maio 2013. Wiley-Blackwell.

MARCHETTI, P. et al. Pancreatic Beta Cell Identity in Humans and the Role of Type 2 Diabetes. **Frontiers In Cell And Developmental Biology,** [s.l.], v. 5, p.1-8, 23 maio 2017. Frontiers Media SA.

MAYNE, R. J.; WOODS, M. G.; LYNCH, G. S. et al. Skeletal effects of the alteration of masseter muscle function. **Australian Orthodontic Journal**, v. 31, n. 2, p.184-194, nov. 2015.

MBATA, O.; EL-MAGD, N. F. A.; EL-REMESSY, A. B.. Obesity, metabolic syndrome and diabetic retinopathy: Beyond hyperglycemia. **World Journal Of Diabetes,** [s.l.], v. 8, n. 7, p.317-331, 2017. Baishideng Publishing Group Inc..

MERCURIO, V. et al. Insulin resistance: Is it time for primary prevention?. **World Journal of Cardiology**, v. 4, n. 1, p.1-7, 2012.

NICOLAIDES, N. C. et al. Circadian endocrine rhythms: the hypothalamic-pituitary-adrenal axis and its actions. **Annals Of The New York Academy Of Sciences,** [s.l.], v. 1318, n. 1, p.71-80, maio 2014. Wiley-Blackwell.

ORGANIZAÇÃO MUNDIAL DE SAÚDE. **Global report on diabetes**. Geneva: OMS, 2016.

PAN, S. Y. et al. Relation of insulin resistance with social-demographics, adiposity and behavioral factors in non-diabetic adult Canadians. **Journal Of Diabetes & Metabolic Disorders,** [s.l.], v. 15, n. 1, p.1-11, dez. 2015. Springer Nature.

PAREDES, S.; ALVES, M.. Abordagem e Tratamento da Hiperglicemia Induzida por Glicocorticóides. **Acta Médica Portuguesa,** [s.l.], v. 29, n. 9, p.556-564, 30 set. 2016. Ordem dos Medicos..

PASIEKA, A.; RAFACHO, A.. Impact of Glucocorticoid Excess on Glucose Tolerance: Clinical and Preclinical Evidence. **Metabolites,** [s.l.], v. 6, n. 3, p.24-45, 3 ago. 2016. MDPI AG.

PREVIS, S. F. et al. Contrasting Effects of IRS-1VersusIRS-2 Gene Disruption on Carbohydrate and Lipid Metabolismin Vivo. **Journal Of Biological Chemistry,** [s.l.], v. 275, n. 50, p.38990-38994, 19 set. 2000. American Society for Biochemistry & Molecular Biology (ASBMB).

PROCÓPIO, A. S. F. et al. Ressecção completa do arco zigomático na fase de crescimento: estudo experimental em ratos. **Pesquisa Odontológica Brasileira,** [s.l.], v. 16, n. 2, p.175-179, jun. 2002. FapUNIFESP (SciELO).

RODRIGUES, L. et al. Effects of the unilateral removal and dissection of the masseter muscle on the facial growth of young rats. **Brazilian Oral Research,** [s.l.], v. 23, n. 1, p.89-95, mar. 2009. FapUNIFESP (SciELO).

SAINI, V.. Molecular mechanisms of insulin resistance in type 2 diabetes mellitus. **World Journal Of Diabetes,** [s.l.], v. 1, n. 3, p.68-76, 2010. Baishideng Publishing Group Inc..

SANDOUK, Z.; LANSANG, M. C.. Diabetes with obesity—Is there an ideal diet? **Cleveland Clinic Journal Of Medicine,** [s.l.], v. 84, n. 71, p.4-14, 1 jul. 2017. Cleveland Clinic Journal of Medicine.

STREHL, C.; BUTTGEREIT, F.. Optimized glucocorticoid therapy: Teaching old drugs new tricks. **Molecular And Cellular Endocrinology,** [s.l.], v. 380, n. 1-2, p.32-40, nov. 2013. Elsevier BV.

SUH, S.; PARK, M.. Glucocorticoid-Induced Diabetes Mellitus: An Important but Overlooked Problem. **Endocrinology And Metabolism,** [s.l.], v. 32, n. 2, p.180-190, 2017. Korean Endocrine Society (KAMJE).

THIRONE, A. C. P.; HUANG, C.; KLIP, A.. Tissue-specific roles of IRS proteins in insulin signaling and glucose transport. **Trends In Endocrinology & Metabolism,** [s.l.], v. 17, n. 2, p.72-78, mar. 2006. Elsevier BV.

VAN DER GOES, M. C. et al. Can adverse effects of glucocorticoid therapy be prevented and treated? **Expert Opinion On Pharmacotherapy,** [s.l.], v. 17, n. 16, p.2129-2133, 20 set. 2016. Informa UK Limited.

VAN RAALTE, D. H.; DIAMANT, M.. Steroid diabetes: from mechanism to treatment?. **The** *Netherlands Journal of Medicine*, v. 72, n. 2, p.62-72, feb. 2014.

WIDMER, C. G.; ENGLISH, A. W.; MORRIS-WIMAN, J.. Developmental and functional considerations of masseter muscle partitioning. **Archives Of Oral Biology,** [s.l.], v. 52, n. 4, p.305-308, abr. 2007. Elsevier BV.

ZHOU, X.; SHENTU, P.; XU, Y.. Spatiotemporal Regulators for Insulin-Stimulated GLUT4 Vesicle Exocytosis. **Journal Of Diabetes Research,** [s.l.], v. 2017, p.1-9, 2017. Hindawi Limited.

ZISMAN A. et al. Targeted disruption of the glucose transporter 4 selectively in muscle causes insulin resistance and glucose intolerance. **Nature Medicine,** [s.l.], v. 6, n. 8, p.924-928, 1 ago. 2000. Springer Nature.

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Letters to the Editor*: Letters must include evidence to support a position about the scientific or editorial content of the *JDR*. Manuscripts submitted as a letter to editor have a limit of 250 words. No figures or tables are permitted. Letters on published articles must be submitted within 3 months of the article's print publication date.

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-Due to the broad readership, abbreviations commonly recognized in one field may not be readily apparent to those in a different field. Keep abbreviation use to a minimum.

-The cover page, abstract, text, summary, figure legends, and tables should be combined into a single Word document. Figures should be submitted as a separate document.

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All submissions must include a title page and be accompanied by a cover letter and list of suggested reviewers. Cover letters should certify the research is original, not under publication consideration elsewhere, and free of conflict of interest. Title pages should include: abstract word count, total word count (Abstract to Acknowledgments), total number of tables/figures, number of references, and a minimum of 6 keywords. Keywords cannot be words that have been included in the manuscript title. Key words should be selected from Medical Subject Headings (MeSH) to be used for indexing of articles. See: http://www.nlm.nih.gov/mesh/MBrowser.html for information on the selection of key words.

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ACKNOWLEDGMENTS

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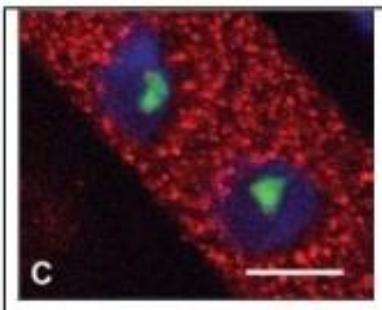
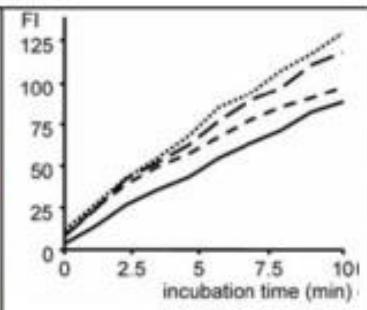
JDR figures can fall into one of three categories: **Continuous-tone images**, **Line-art images**, and **Combination images.** Each image type has specific requirements in terms of the resolution needed for publication and the file types best suited for the figure. See the following panels for examples and requirements.



Continuous-tone Image
Minimum resolution: 300dpi.
Preferred File Formats: TIFF, Bitmap.



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Minimum resolution: 800dpi.
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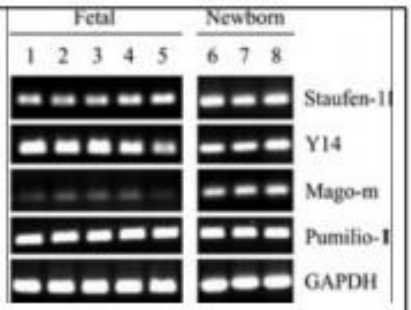

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PowerPoint, Illustrator, InDesign.

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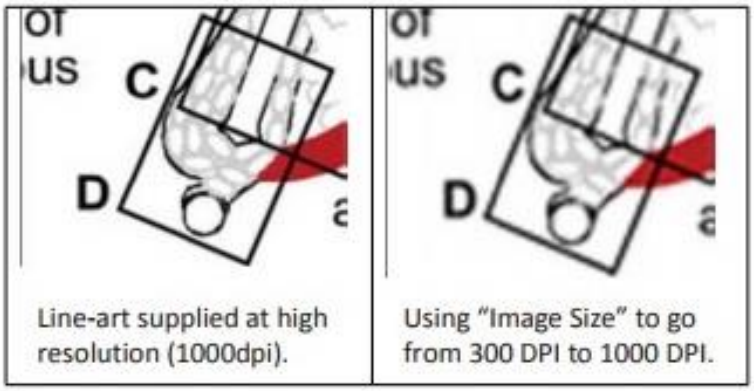
In order for a figure to be used in publication, its Digital Image File must have the required resolution when it is created. The resolution cannot be raised *after* the original image is made. Attempting to do so (for example, with Adobe Photoshop's© *"Image Size"* command) results in the addition of artificial pixels that distort the image and lower its sharpness. The figures on the right show an example of this reduced sharpness.



Line-art supplied at high resolution (1000dpi).

Using "Image Size" to go from 300 DPI to 1000 DPI.

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III- ARTIGO ORIGINAL

Insulin signaling pathway in the masseter muscle of dexamethasone-treated rats

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Abstract

Insulin resistance is partly caused by molecular changes in the level and/or degree of phosphorylation of proteins located downstream of the insulin receptor/insulin-like growth factor receptor (IR/IGF1R) signaling pathway that occurs in the skeletal muscle, liver, and adipose tissue. However, few studies have investigated the intracellular insulin pathway in the masseter muscle under altered metabolic conditions such as obesity and diabetes. Therefore, this study aimed to analyze the IR/IGF1R signaling pathway in the masseter muscle of rats treated with dexamethasone (a glucocorticoid that induces manifestations of insulin resistance at high concentrations). Male Wistar rats were divided into two groups: control group, intraperitoneally injected with 0.9% NaCl (saline solution), and dexamethasone group, intraperitoneally injected with a dexamethasone solution (1 mg/kg body weight) for 10 consecutive days. Sections of the masseter muscle were removed at time zero and after the infusion of regular insulin into the portal vein. The administration of dexamethasone induced body weight loss without changing the masseter muscle weight. In addition, it reduced the expression of total IR and PI3K proteins, with the total levels of IRS1, AKT, and ERK1 remaining unchanged compared with the levels in the control group. The degree of phosphorylation/activity of IRS1 after insulin stimulus increased in the control group but not in the dexamethasone group. The degree of phosphorylation of AKT increased in both groups, but this increase was attenuated in the dexamethasone group. We suggest that the degree of phosphorylation/activity in the masseter muscle is different from that in other muscle territories.

Keywords: masseter muscle; dexamethasone; insulin receptor

1. Introduction

Changes in lifestyle in recent decades have altered the metabolic profile of the world's population (Kelly et al. 2008; Sandouk and Lansang 2017). The increase in the number of overweight or obese individuals as a result of excessive consumption of hypercaloric foods, sedentarism, and low intake of fibers has contributed to the morbimortality of populations, with an increased prevalence of diabetes mellitus type 2, high blood pressure, dyslipidemia, peripheral resistance to insulin (InsR), and other disorders that characterize metabolic syndrome (Branddon et al. 1986; Kelly et al. 2008; Guh et al. 2009; Capehorn et al. 2016).

Among these metabolic changes, InsR is a clinical condition characterized by a decreased response to the action of insulin in target organs, which results in high serum levels of glucose associated with compensatory hyperinsulinemia through an increase in the number of beta-pancreatic cells (DeFronzo and Ferrannini 1991; Rafacho et al. 2010; DeFronzo et al. 2015; Kim et al. 2016).

InsR in peripheral tissues (adipose tissue, liver, and skeletal muscle) is partly caused by molecular adjustments in the level and/or degree of phosphorylation of proteins involved in the intracellular insulin receptor/insulin-like growth factor receptor (IR/IGF1R) signaling pathway (Velloso et al. 1995; Saltiel and Kahn 2001; Kahn and Pessin 2002; Kulkarni 2002; Houmard et al. 2012; Zhou et al. 2017).

Proteins located downstream of the IR/IGF1 signaling pathway can be phosphorylated at tyrosine residues of IR substrates (IRSs), mainly IRS1 and IRS2. IRS1 is involved in the stimulation of glucose uptake and glycogen storage in the skeletal muscle, whereas IRS2 acts on the metabolism of the skeletal muscle, adipose tissue, and liver (Previs et al. 2000; Thirone et al. 2006). The phosphorylation of these substrates leads to the activation of several kinases, namely phosphatidylinositol 3-kinase (PI3K), which causes the activation of protein kinase B (PKB or AKT) through phosphorylation at serine residues. AKT has several roles: glucose transportation, glycogen synthesis, protein synthesis, lipogenesis, and liver gluconeogenesis inhibition (DeFronzo et al. 2015; Zhou et al. 2017).

Dexamethasone, which is a potent synthetic glucocorticoid with dose-dependent anti-inflammatory and immunosuppressant activities, has been used to investigate intracellular insulin signaling pathways during InsR (Rafacho et al. 2010; Gathercole et al. 2011; Strehl and Buttgereit 2013). In addition to InsR, the adverse effects of its chronic use include dyslipidemia, increased localized fat, and muscle deterioration, which are manifestations that are similar to

those in Cushing's syndrome (Christ-Crain et al. 2008; Van Raalte et al. 2009; Barel et al. 2009; Umeki et al. 2015; Tsuchida et al. 2016).

Glucocorticoids can decrease the expression of IRS1, PI3K, and PKB, causing the migration of a reduced amount of GLUT4 (characteristically found in the skeletal muscle) toward the membrane, thus decreasing glucose uptake and glycogen synthesis (Ruzzin et al. 2005; Gholap and Kar 2005; Zhou et al. 2017).

Among the tissues that respond to insulin, the skeletal muscle accounts for approximately 75% of glucose uptake as it is the largest muscle in the human body (Houmard et al. 2012). Various skeletal muscle territories, such as the tibialis anterior, extensor digitorum longus (Barel et al. 2009), soleus, epitrochleoanconeus (Anhe et al. 2007; Burén et al. 2008), and gastrocnemius (Okamoto et al. 2011) muscles, have been used as experimental models to assess the intracellular insulin signaling pathway under different metabolic conditions (DeFronzo and Tripathy 2009). However, this pathway has not yet been fully understood in the masseter muscle. Therefore, the present study aimed to analyze IR/IGF1/IRS1/AKT/PI3K in the masseter muscle under normal conditions and in the presence of InsR induced by dexamethasone.

2. Materials and methods

Animal Model

Animal procedures were conducted according to the guidelines of the Ethics Committee on Animal Research of the Federal University of Sergipe. The study was approved by the Ethics Committee on Animal Research of the Federal University of Sergipe under protocol (CEPA 54/2009). Male Wistar rats were used from the central animal house of the Federal University of Sergipe, São Cristóvão, Sergipe, Brazil, and they weighed between 250 and 300 g. The rats were kept in a controlled environment (temperature of 23 ± 2°C and light/dark cycle of 12-h light period between 6:00 (zeitgeber time [ZT0]) and 18:00 (zeitgeber time [ZT12]) and were offered commercial feed for rodents (Ração Labina, São Paulo, SP) and water *ad libitum*. 16 rats were randomly divided into two groups: control group (CON group), intraperitoneally injected with 0.9% NaCl (saline solution), and dexamethasone group (DEX group), intraperitoneally injected with 1 mg/kg body weight of dexamethasone (Decadron® Aché, Guarulhos-SP), in both groups for 10 consecutive days during the ZT6 period (12:00). The body weight of the rats was measured on a daily basis. On the 11th day, the rats were euthanized with

the anesthetic sodium thiopental (Thiopentax®, Cristália, Itapira-SP) (40 mg/kg body weight) and their body weight and masseter muscle weight were measured for subsequent statistical analyses.

Protein Quantification by Western Blotting

Sections of the right masseter muscle were isolated to establish time zero (basal protein phosphorylation). Subsequently, 0.5 ml of saline solution containing 5 mmol/l of insulin per kg of body weight was injected into the portal vein, and after 3 min, the sections of the left masseter muscle were removed from all rat in both groups. The sections were macerated and transferred to Eppendorf tubes and homogenized in buffer solutions (Tris: 100 mM pH 7.4; EDTA: 10 mM; SDS 1%, $Na_2P_2O_7$: 100 mM; NaF: 100 mM and Na_2VO_4: 10 mM). Extracts were centrifuged at 12,000 rpm for 40 min at 4°C to remove the insoluble material. Protein content in the supernatant was quantified using the Bradford protein assay (BioRad) and treated with Laemmli buffer; 25–50 μg of total protein was separated by polyacrylamide gel electrophoresis (SDS-PAGE 6.5% and 12% polyacrylamide) and transferred to 0.45-μm nitrocellulose membranes. The membranes were blocked for 1 h in 5% nonfat milk. Subsequently, the samples were incubated overnight at 8°C with antibodies against IR, IRS1, PI3K, AKT, pAKT (serine residue 473), and ERK1 diluted in a blocking buffer containing 3% nonfat dry milk. They were then washed without milk for 30 min with the blocking buffer. Lastly, the intensities of the bands were quantified by optical densitometry (Scion Image-Release Beta 3b; NIH), and Ponceau S staining was performed to obtain a loading control. All antibodies were purchased from Santa Cruz Biotechnology.

Statistical Analysis

All results are expressed as mean ± SEM. Two-way ANOVA followed by Bonferroni's post hoc test was used to compare the two groups. Student's t-test was used whenever necessary. The level of significance was set at $p < 0.05$. All statistics and graphs were analyzed using Prism software for Windows, version 5.01 (GraphPad Software, San Diego, CA, USA).

3. Results

Body weight gain and wet weight/dry weight ratio

The body weight of the animals at the start of the study was similar between the groups. At the end of the final protocol (after 10 days of treatment), the animals in the DEX group exhibited a body weight loss of 15.42% ($p < 0.05$), whereas the animals in the CON group exhibited a body weight gain of 7.99% ($p < 0.05$) (Figure 1A). The wet and dry weights (g) of the masseter muscle did not statistically differ between the groups (Figures 1B and 1C). The ratio between the wet weight of the masseter muscle and body weight after treatment was 16.87% higher in the DEX group than in the CON group (Figure 1D).

Degree of expression of IR, IRS1, and PI3K and degree of phosphorylation/activity of IRSs and AKT

The degree of expression of IR, IRS1, and PI3K obtained from sections of the masseter muscle of the animals in the DEX group. The analysis of the intensity of bands in the membranes by densitometry showed that the levels of IR (Figure 2A) and PI3K (Figure 2B) in the masseter muscle were 56.85% and 30.73%, respectively, lower in the DEX group than in the CON group ($p < 0.05$). The total level of IRS1 was similar between the groups (Figure 2C). Figure 2D shows the degree of phosphorylation/activity of IRS1 obtained from sections of the masseter muscle of animals in the DEX and CON groups at the start of the experiment [time zero, (–)] and after insulin stimulus (+). After insulin stimulus, the degree of phosphorylation/activity of tyrosine residues of IRS1 in the masseter muscle of the animals in the CON group was 38.15% higher than that at time zero (−) ($p < 0.05$). However, in the DEX group, there was no difference in the degree of phosphorylation before and after insulin stimulus (Figure 2D). The total level of AKT in the masseter muscle (Figure 2E) was similar between the groups. Figure 2F shows the degree of phosphorylation/activity of AKT (phosphorylated at serine 473) obtained from sections of the masseter muscle of animals in the CON and DEX groups at time zero (–) and after insulin stimulus (+). After insulin stimulus, the degree of phosphorylation/activity of AKT from sections of the masseter muscle in the CON group was 513.18% higher than that at time zero (−) ($p < 0.05$). The DEX group also showed an increase

in AKT phosphorylation compared with basal phosphorylation, but the magnitude of this increase was attenuated after insulin stimulus (266.63%) ($p < 0.05$) (Figure 2F).

Degree of ERK1 expression

The expression of ERK1 obtained from sections of the masseter muscle of animals in the DEX and control groups. The analysis of the intensity of bands in membranes by densitometry indicated significant differences between the groups (Figure 3).

4. Discussion

In the present study, treatment with dexamethasone induced body weight loss, which is in line with literature data associated with reduced food intake and glucocorticoid dose-dependent weight (Jahng et al. 2008). Similar studies have demonstrated that treatment with dexamethasone at 1 mg/kg body weight leads to body weight loss, muscular atrophy, and InsR as shown in the anterior tibialis and extensor digitorum longus muscles (Barel et al. 2009). However, the present study did not show any significant difference in the wet and dry weights of the masseter muscle.

The ratio between masseter muscle weight and body weight was significantly higher in the DEX group. Studies have shown that muscular atrophy caused by high doses and/or the chronic use of dexamethasone is a consequence of a reduced rate of muscle protein synthesis and an increased rate of muscle proteolysis, which is a result of the impairment of several kinases of the insulin signaling pathway (Egerman and Glass 2013; Tsuchida et al. 2016).

Dexamethasone, which is a potent synthetic glucocorticoid analog of cortisol, was used to induce InsR. It is widely used in the field of pharmacology for its dose-dependent anti-inflammatory and immunosuppressive activities (Strehl and Buttgereit 2013; Pasieka and Rafacho 2016). Moreover, its chronic use induces adverse effects in the body, such as skeletal muscle myopathy (Barel et al. 2009; Umeki et al. 2015) and muscular weakness and deterioration in patients with Cushing's syndrome (Tsuchida et al. 2016).

Few studies in the literature have investigated the insulin signaling pathway in the masseter muscle, which is a complex muscle that covers almost the entire side of the jaw and is thus involved in mastication, salivation, and speech. In male rats, masseter muscle fibers exhibit an intracellular response to the action of testosterone, which in turn promotes the phenotypic transformation of part of the slow fibers into fast fibers (Widmer et al. 2007).

According to these authors, the predominance of fast fibers in this muscle appears to be associated with the increase in force generation during mastication.

In the present study, total IR expression was lower in the DEX group than in the CON group, which may result in changes in the activity or degree of phosphorylation of intracellular proteins located downstream of the IR/IGF1R signaling pathway. Gathercole et al. (2007) reported that there was an increase in the degree of IRS-1 phosphorylation in primary cultures of skeletal muscle myocytes incubated with dexamethasone for 48 h. Saad et al. (1993) suggested that dexamethasone induces InsR in the skeletal muscle, with a decrease in the total IRS1 content associated with an increase in the degree of phosphorylation in muscles of the posterior limbs of rats. In the present study, there was no significant difference in total IRS1 content after treatment with dexamethasone.

There was an increase in the degree of phosphorylation/activity of IRS1 in the masseter muscle of the control animals after insulin stimulus but not in the muscle of the animals treated with dexamethasone. Our results differed from those obtained by Saad et al. (1993) who investigated the leg's hindlimb muscle. We do not exclude the hypothesis that proteins involved in the IR/IGF1R signaling pathway have distinct levels and/or degrees of phosphorylation because they exhibit tissue-specific responses. Burén et al. (2008) stated that the degree of phosphorylation of proteins involved in the IR/IGF1R signaling pathway isolated from fast muscle fibers is different from that of fibers in other muscle territories, such as the soleus muscle.

The masseter muscle of the rats treated with dexamethasone had a lower total PI3K level than that of the control rats. PI3K is involved in the metabolism of glucose stimulated by insulin and acts on mitogenesis and cell differentiation (Saltiel and Kahn 2001; Glass 2010). The phosphorylation of PI3K in turn increases the degree of phosphorylation of the serine residues of PKB (also known as AKT). AKT is an important central component of the signaling pathway and is involved in protein synthesis (via the activation of mammalian target of rapamycin kinase and of the glycogen synthase kinase 3β pathway) (Schiaffino and Mammucari 2011; Egerman and Glass 2013) and in intracellular protein degradation (mediated by the transcription of proteins belonging to the FoxO family) (L`eger et al. 2006; Bacurau et al. 2016).

Although in the present study there was no significant change in the expression of total AKT, there was an increase in the degree of AKT phosphorylation in the CON group after insulin stimulus. The increase in the degree of phosphorylation was attenuated in the DEX group after insulin stimulus. According to Umeki et al. (2015), treatment with dexamethasone for two weeks promotes atrophy of the masseter muscle and these events are related to the

downregulation of the AKT/mTOR pathway and upregulation of the proteolytic pathway (MURF1 and ATROGIN). These effects are probably caused by the high glucocorticoid concentration that was used (six times higher than that used in the present study).

Previs et al. (2000) reported the involvement of different isoforms of IRSs, e.g., IRS1 and IRS2, that when phosphorylated act differently on different organs and on body development. They demonstrated that the deletion of IRS1 in the body causes delayed growth and contributes to the development of InsR. The total deletion of IRS2 induces the development of diabetes, beta-pancreatic cell dysfunction, and peripheral and hepatic InsR. A review conducted by Thirone et al. (2006) on the ERK/MAPK pathway also showed that these substrates have distinct actions on the skeletal muscle. IRS2 appears to act directly on the AKT/ERK/p38MAPK-dependent mitogenic and metabolic pathways, whereas IRS1 is implicated in events leading to the translocation of vesicles containing GLUT4 to the plasma membrane and in glucose uptake. In the present study, the total level of ERK1 in the masseter muscle was similar between the groups; as no atrophy of the masseter muscle was observed, we suggest that the IRS2 pathway was unchanged.

In conclusion, treatment with dexamethasone promoted the reduction in the total amount of IR and PI3K. The degrees of phosphorylation of IRS1 and AKT were altered without implications for the total weight of the masseter muscle. These results indicate that the masseter muscle, because it is composed of fast fibers, exhibits a degree of phosphorylation/activity that is different from that of fibers in other muscle territories. In addition, the role of other pathways, such as those mediated by IRS2/AKT/ERK/p38MAPK, in the preservation of this muscle should not be ruled out.

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References

Anhe GF, Hirabara SM, Turrer TC, Caperuto LC, Anhe FF, Ribeiro LM, Marçal AC, Carvalho CROC, Machado UF, Borfin S, et al. 2007. Postpartum glycemic homeostasis in early lactating rats is accompanied by transient and specific increase of soleus insulin response through IRS2/AKT pathway. Ajp: Regulatory, Integrative and Comparative Physiology. 292(6):2225-2233.

Bacurau AV, Cunha TF, Souza RW, Voltarelli VA, Gabriel-Costa D, Brum PC. 2016. Aerobic Exercise and Pharmacological Therapies for Skeletal Myopathy in Heart Failure: Similarities and Differences. Oxidative Medicine And Cellular Longevity. 2016:1-16.

Barel M, Perez OAB, Giozzet VA, Rafacho A, Bosqueiro JR, Amaral SL. 2009. Exercise training prevents hyperinsulinemia, muscular glycogen loss and muscle atrophy induced by dexamethasone treatment. European Journal Of Applied Physiology. 108(5):999-1007.

Braddon FEM, Rodgers B, Wadsworth MEJ, Davies JMC. 1986. Onset of obesity in a 36 year birth cohort study. Bmj. 293(6542):299-303.

Burén J, Lai YC, Lundgren M, Eriksson JW, Jensen J. 2008. Insulin action and signalling in fat and muscle from dexamethasone-treated rats. Archives Of Biochemistry And Biophysics. 474(1):91-101.

Capehorn MS, Haslam DW, Welbourn R. 2016. Obesity Treatment in the UK Health System. Current Obesity Reports. 5(3):320-326.

Christ-Crain M, Kola B, Lolli F, Fekete C, Seboek D, Wittmann G, Feltrin D, Igreja SC, Grossman AB, Korbonits M, et al. 2008. AMP-activated protein kinase mediates glucocorticoid-induced metabolic changes: a novel mechanism in Cushing's syndrome. The Faseb Journal. 22(6):1672-1683.

DeFronzo RA, Ferrannini E. 1991. Insulin Resistance: A Multifaceted Syndrome Responsible for NIDDM, Obesity, Hypertension, Dyslipidemia, and Atherosclerotic Cardiovascular Disease. Diabetes Care. 14(3):173-194.

DeFronzo RA, Tripathy D. 2009. Skeletal Muscle Insulin Resistance Is the Primary Defect in Type 2 Diabetes. Diabetes Care. Diabetes Care. 32(2):157-163.

DeFronzo RA, Ferrannini E, Groop L, Henry RR, Herman WH, Holst JJ, Hu FB, Kahn CR, Raz I, Shulman GI, et al. 2015. Type 2 diabetes mellitus. Nature Reviews Disease Primers. 1:15019-15019.

Egerman MA, Glass DJ. 2013. Signaling pathways controlling skeletal muscle mass. Critical Reviews In Biochemistry And Molecular Biology. 49(1):59-68 [s.l.], v. 49, n. 1, p.59-68, 18 nov. 2013. Informa UK Limited.

Gathercole LL, Bujalska IJ, Stewart PM, Tomlinson JW. 2007. Glucocorticoid Modulation of Insulin Signaling in Human Subcutaneous Adipose Tissue. The Journal Of Clinical Endocrinology & Metabolism. 92(11):4332-4339.

Gathercole LL, Morgan SA, Bujalska IJ, Hauton D, Stewart PM, Tomlinson JW. 2011. Regulation of Lipogenesis by Glucocorticoids and Insulin in Human Adipose Tissue. Plos One. 6(10):26223-26231.

Gholap S, Kar A. 2005. Gymnemic Acids fromGymnema sylvestre. Potentially Regulates Dexamethasone-Induced Hyperglycemia in Mice. Pharmaceutical Biology. 43(2):192-195.

Glass DJ. 2010. PI3 Kinase Regulation of Skeletal Muscle Hypertrophy and Atrophy. Current Topics In Microbiology And Immunology. 1:267-278.

Guh DP, Zhang W, Bansback N, Amarsi Z, Birmingham L, Anis AH. 2009. The incidence of co-morbidities related to obesity and overweight: A systematic review and meta-analysis. Bmc Public Health. 9(1):1-20.

Houmard JA, Pories WJ, Dohm GL. 2012. Severe Obesity: Evidence for a Deranged Metabolic Program in Skeletal Muscle?. Exercise And Sport Sciences Reviews. 40(4):204-210.

Jahng JW, Kim NY, Ryu V, Yoo SB, Kim BT, Kang DW, Lee JH. 2008. Dexamethasone reduces food intake, weight gain and the hypothalamic 5-HT concentration and increases plasma leptin in rats. European Journal Of Pharmacology. 581(1-2):64-70.

Kelly T, Yang W, Chen CS, Reynolds K, He J. 2008. Global burden of obesity in 2005 and projections to 2030. International Journal Of Obesity. 32(9):1431-1437.

Khan A, Pessin J. 2002. Insulin regulation of glucose uptake: a complex interplay of intracellular signalling pathways. Diabetologia. 45(11)1475-148.

Kim MK, Reaven GM, Kim SH. 2016. Dissecting the relationship between obesity and hyperinsulinemia: Role of insulin secretion and insulin clearance. Obesity. 25(2):378-383.

Kulkarni RN. 2002. Receptors for insulin and insulin-like growth factor-1 and insulin receptor substrate-1 mediate pathways that regulate islet function. Biochemical Society Transactions. 30(2):317-322.

L`eger B, Cartoni R, Praz M, Lamon S, D`eriaz O, Crettenand A, Gobeleti C, Rohmer P, Konzelmann M, Luthi F et al. 2006. Akt signalling through GSK-3β, mTOR and Foxo1 is involved in human skeletal muscle hypertrophy and atrophy. The Journal Of Physiology. 576(3):923-933.

Okamoto MM, Anhê GF, Sabino-Silva R, Marques MFDSF, Freitas HS, Mori RCT, Melo KFS. 2011. Intensive insulin treatment induces insulin resistance in diabetic rats by impairing glucose metabolism-related mechanisms in muscle and liver. Journal of Endocrinology. 211(1):55–64.

Pasieka A, Rafacho A. 2016. Impact of Glucocorticoid Excess on Glucose Tolerance: Clinical and Preclinical Evidence. Metabolites. 6(3):24-24.

Previs SF, Withers DJ, Ren J, White MF, Shulman GI. 2000. Contrasting Effects of IRS-1 Versus IRS-2 Gene Disruption on Carbohydrate and Lipid Metabolism in Vivo. Journal of Biological Chemistry. 275(50):38990–4.

Rafacho A, Marroquí L, Taboga SR, Abrantes LF, Silveira LR, Boschero AC, Carneiro EM, Bosqueiro R, Nadal A, Quesada I. 2010. Glucocorticoids in Vivo Induce Both Insulin Hypersecretion and Enhanced Glucose Sensitivity of Stimulus-Secretion Coupling in Isolated Rat Islets. Endocrinology. 151(January):85–95.

Ruzzin J, Wagman AS, Jensen J. 2005. Glucocorticoid-induced insulin resistance in skeletal muscles: defects in insulin signalling and the effects of a selective glycogen synthase kinase-3 inhibitor. Diabetologia. 48(10):2119-2130.

Saad MJA, Folli F, Kahn JA, Kahn CR. 1993. Modulation of insulin receptor, insulin receptor substrate-1, and phosphatidylinositol 3-kinase in liver and muscle of dexamethasone-treated rats. Journal Of Clinical Investigation, 92(4):2065-2072.

Saltiel AR, Kahn CR. 2001. Insulin signalling and the regulation of glucose and lipid metabolism. Nature. 414(6865):799–806.

Sandouk Z, Lansang MC. 2017. Diabetes with obesity—Is there an ideal diet? Cleveland Clinic Journal Of Medicine. 84(71):4-14.

Schiaffino S, Mammucari C. 2011. Regulation of skeletal muscle growth by the IGF1-Akt/PKB pathway: insights from genetic models. Skeletal Muscle 1(1):4-18.

Strehl C, Buttgereit F. 2013. Optimized glucocorticoid therapy: Teaching old drugs new tricks. Molecular And Cellular Endocrinology. 380(1-2):32-40.

Thirone AC, Huang C, Klip A. 2006. Tissue-specific roles of IRS proteins in insulin signaling and glucose transport. Trends In Endocrinology & Metabolism. 17(2):72-78.

Tsuchida W, Iwata M, Akimoto T, Matsuo S, Asai Y. 2016. Heat Stress Modulates Both Anabolic and Catabolic Signaling Pathways Preventing Dexamethasone-Induced Muscle Atrophy In Vitro. Journal Of Cellular Physiology. 232(3):650-664.

Umeki D, Ohnuki Y, Matotani Y, Shiozawa K, Suita K, Fukita T, Nakamura Y, Saeki Y, Okumura S. 2015. Protective Effects of Clenbuterol against Dexamethasone-Induced Masseter Muscle Atrophy and Myosin Heavy Chain Transition. Plos One. 10(6).

Van Raalte DH, Ouwens DM, Diamant M. 2009. Novel insights into glucocorticoid-mediated diabetogenic effects: towards expansion of therapeutic options?. European Journal Of Clinical Investigation. 39(2)81-93.

Velloso LA, Carneiro EM, Crepaldi SC, Boschero AC, Saad MJA. 1995. Glucose- and insulin-induced phosphorylation of the insulin receptor and its primary substrates IRS-1 and IRS-2 in rat pancreatic islets. FEBS Lett. 377(3):353-7.

Zhou X, Shentu P, Xu Y. 2017. Spatiotemporal Regulators for Insulin-Stimulated GLUT4 Vesicle Exocytosis. Journal Of Diabetes Research. 2017: 1-9.

Widmer CG., English AW, Morris-Wiman J. 2007. Developmental and functional considerations of masseter muscle partitioning. Archives Of Oral Biology. 52(4):305-308.

Figure legends:

Figure 1: Assessment of body weight and masseter muscle weight (wet weight and dry weight) in the CON group (hollow bars; n = 10) and DEX group (solid bars; n = 10). 1A: Analysis of body weight in the CON and DEX groups at the start of dexamethasone treatment (time zero) and after 10 days of treatment. **1B:** Analysis of wet weight (g) of the masseter muscle in the CON and DEX groups after 10 days of treatment. **1C:** Analysis of dry weight (g) of the masseter muscle in the CON and DEX groups after 10 days of treatment. **1D:** Ratio between the wet weight of the masseter muscle and body weight in the CON and DEX groups. Student's t-test was used for the analysis between the groups ($p < 0.05$).

Figure 2: Samples containing 25–50 mg of solubilized proteins were subjected to SDS-PAGE and immunoblotting using specific antibodies. A blot representative of the experiments is shown. The status of phosphorylation and protein expression (percentage) involved in intracellular insulin signaling in the masseter muscle of rats in the CON group (hollow bars; n = 6) and DEX group (solid bars; n = 6) was determined by stoichiometry. Analysis of the degree of expression of IR **(2A)**, PI3K **(2B)**, and IRS1 **(2C)** in the masseter muscle in the CON and DEX groups. Analysis of the degree of IRS1 phosphorylation/activity in the masseter muscle in the CON and DEX groups before [time zero, (−)] and after the infusion of insulin into the portal vein (+) (**2D)**. Total amount of AKT protein in the masseter muscle in the CON and DEX groups **(2E)**. Analysis of the degree of phosphorylation/activity (phosphorylation at serine 473) of AKT in the masseter muscle in the CON and DEX groups before [time zero, (−)] and after the infusion of insulin into the portal vein (+) **(2F)**. Student's t-test was used in the intergroup analysis ($p < 0.05$).

Figure 3: Analysis of the degree of ERK1 expression in the masseter muscle of animals in the CON group (hollow bars; n = 6) and DEX group (solid bars; n = 6). Student's t-test was used in the analysis between the groups ($p < 0.05$).

Figura 1

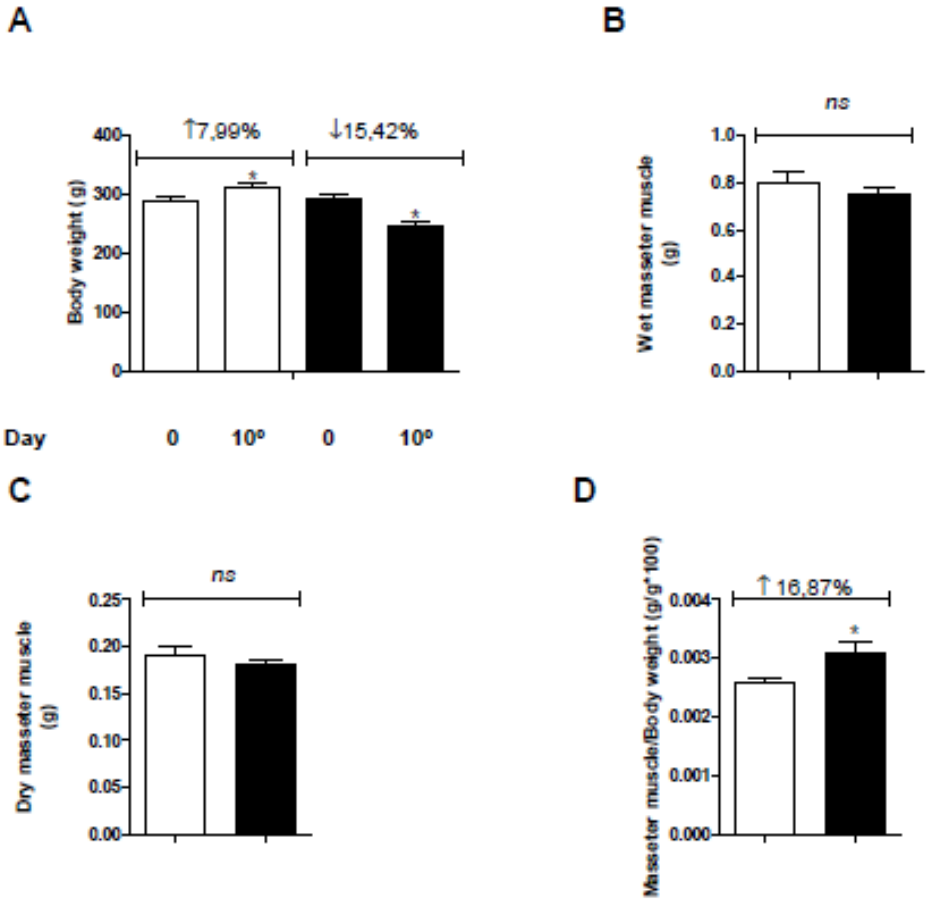

A
B
↑7,99%
↓15,42%
Body weight (g)
400
300
200
100
0
*
*
Day
0
10º
0
10º
ns
Wet masseter muscle (g)
1.0
0.8
0.6
0.4
0.2
0.0
C
D
ns
Dry masseter muscle (g)
0.25
0.20
0.15
0.10
0.05
0.00
↑ 16,87%
Masseter muscle/Body weight (g/g*100)
0.004
0.003
0.002
0.001
0.000
*

Figura 2

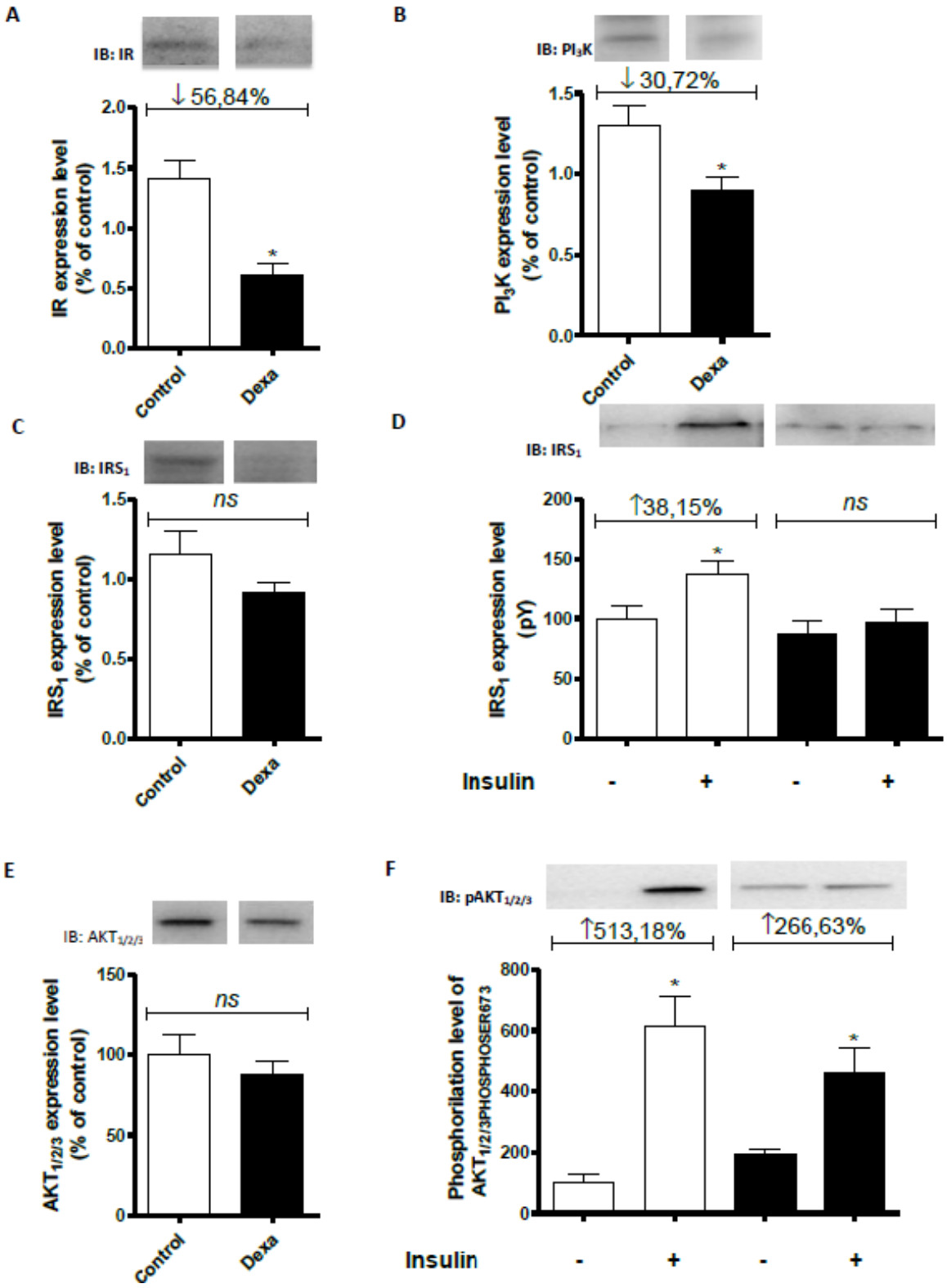
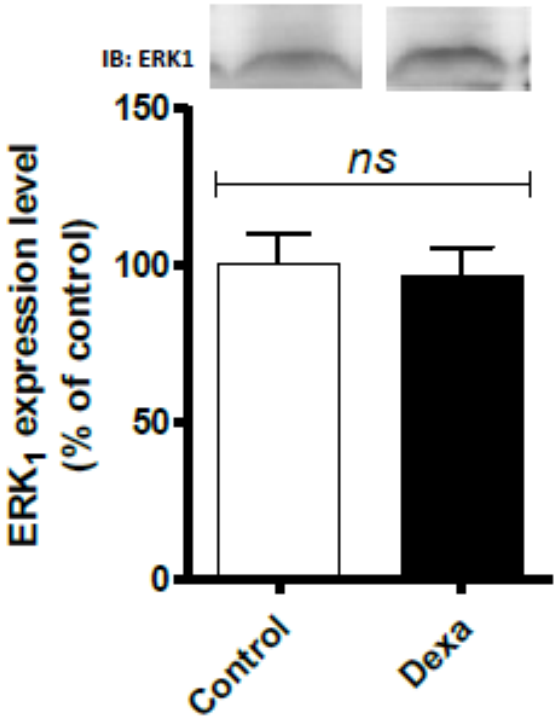

A
IB: IR
↓56,84%
IR expression level (% of control)
Control
Dexa
B
IB: PI3K
↓30,72%
PI3K expression level (% of control)
Control
Dexa
C
IB: IRS1
ns
IRS1 expression level (% of control)
Control
Dexa
D
IB: IRS1
↑38,15%
ns
IRS1 expression level (pY)
Insulin - + - +
E
IB: AKT1/2/3
ns
AKT1/2/3 expression level (% of control)
Control
Dexa
F
IB: pAKT1/2/3
↑513,18%
↑266,63%
Phosphorilation level of AKT1/2/3PHOSPHOSER673
Insulin - + - +

Figura 3


IB: ERK1
150
100
50
0
ns
ERK1 expression level
(% of control)
Control
Dexa

IV- ANEXOS

Anexo B: Carta de confirmação de submissão

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Submission Confirmation

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Date Submitted
13-Sep-2017

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Anexo B: Protocolo do Comitê de Ética em Pesquisa com Animais da Universidade Federal de Sergipe (CEPA 54/2009)

UNIVERSIDADE FEDERAL DE SERGIPE
CENTRO DE CIÊNCIAS BIOLÓGICAS E DA SAÚDE
COMITÊ DE ÉTICA EM PESQUISA COM ANIMAIS (CEPA)

DECLARAÇÃO

Declaro, para os devidos fins, que o Projeto de Pesquisa intitulado "Regulação da síntese de melatonina pela glândula pineal: características funcionais e mecanismos moleculares em condições de resistência a ação de insulina", sob coordenação do Prof. Anderson Carlos Marçal (protocolo CEPA 54/2009), foi aprovado pelo Comitê de Ética em Pesquisa com Animais da Universidade Federal de Sergipe, em reunião realizada dia 22/04/2010.

São Cristóvão, 23 de abril de 2010

Profª. Drª. Flavia Teixeira Silva
Presidente do CEPA/UFS

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