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Bárbara Jonson Bartikoski

O PAPEL DOS FATORES DE CRESCIMENTO E DIFERENCIAÇÃO NA PERDA MUSCULAR EM MODELO DE ARTRITE INDUZIDA POR COLÁGENO

Porto Alegre

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(Orientador)

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RESUMO

A artrite reumatoide (AR) é uma doença inflamatória auto-imune, caracterizada por sinóvia com infiltração de leucócitos, resultando em hiperplasia sinovial, degradação da cartilagem e erosão óssea, o que pode gerar déficits musculares nos indivíduos afetados pela doença. Com o objetivo de entender melhor os mecanismos moleculares da perda de massa muscular na AR, os ligantes da família TGF-β, mais especificamente o GDF-8 e o GDF-11, que atuam como reguladores negativos do crescimento muscular, podem desempenhar um papel importante na perda muscular e influenciar a perda de apetite através regulamentação de GDF-15. Portanto, o objetivo deste estudo foi avaliar os níveis musculares de GDF-8, GDF-11 e GDF-15 durante todo o desenvolvimento da artrite experimental (CIA). Camundongos DBA1/J foram submetidos ao modelo de artrite induzida por colágeno (CIA). Camundongos machos, com 8-12 semanas de idade, foram randomizados em três grupos experimentais: animais saudáveis (HA, n=6), animais controles 25 dias, sem qualquer intervenção (CO, n=16) e animais com artrite induzida por colágeno (CIA, n=16). Os animais foram eutanaziados nos dias zero, 25 e 50 após a indução da doença. O teste de força de preensão foi aplicado aos 0. 18, 25 e 50 dias após a indução da doença e o escore e o edema da pata traseira foram medidos a cada 3 dias. As articulações tíbio-tarso foram processadas para confirmação do desenvolvimento da doença. O músculo tibial anterior foi pesado e processado para medir a área transversal da miofibra e a razão sarcoplasmática; o músculo gastrocnêmio foi pesado e congelado para relação sarcoplasmática e expressão de GDF-11, GDF-8 e GDF-15 via reação em cadeia da polimerase (PCR). A análise estatística foi realizada com o SPSS e os resultados foram considerados significativos quando $p<0.05$. O grupo CIA apresentou escores significativamente maiores de artrite e maiores volumes de edema da pata traseira do que o grupo CO na doença inicial e na doença estabelecida (dias 25 e 50 após a indução). O grupo CIA diminuiu a força de preensão em ambos os momentos em relação ao CO. As relações sarcoplasmáticas e o peso muscular também foram reduzidos no grupo CIA na doença estabelecida. O diâmetro da miofibra do músculo tibial anterior apresentou redução no grupo CIA na doença estabelecida em comparação com o CO. Os níveis de GDF-11 foram significativamente maiores no grupo CIA na doença inicial e apresentaram tendência de aumento na doença estabelecida. A expressão de GDF-8 diminuiu em doença estabelecida e GDF-15 não diferiu entre os grupos. Foi encontrada uma correlação negativa entre força muscular e GFD-11 na doença inicial. Portanto, na artrite inicial, a

expressão gênica de GDF-11 é aumentada e também associada à perda de força de preensão, enquanto a expressão gênica de GDF-8 é reduzida na doença estabelecida, possivelmente como um mecanismo compensatório. Assim, os GDFs podem ter um papel nos deficits musculares no modelo da CIA e podem estar envolvidos na atrofia muscular e na perda de força.

Palavras-chave: Perda muscular, artrite reumatoide, GDF-8, GDF-11, GDF-15.

ABSTRACT

Rheumatoid arthritis (RA) is an autoimmune, inflammatory disease characterized by synovium with leukocyte infiltration, resulting in synovial hyperplasia, cartilage degradation and bone erosion, which can generate muscle deficits on disease affected individuals. Aiming to better understand the molecular mechanisms of muscle wasting in RA, TGF-β family ligands, more specifically GDF-8 and GDF-11, that act as negative regulators of muscle growth may play an important role in muscle loss and influence appetite loss through down regulation of GDF-15. So, the aim of this study was to evaluate muscle levels of GDF-8, GDF-11 and GDF-15 throughout the development of experimental arthritis (CIA). DBA1/J mice were submitted to collagen-induce arthritis. Male DBA/1J mice, between 8 and 12 weeks of age, were randomly divided into three experimental groups: healthy animals (HA, n=6), control animals without intervention (CO, n=16) and collagen-induced arthritis animals (CIA, n=16). During the experimental period, disease score and edema, and grip strength were evaluated. Mice were euthanized at day 0. 25 or 50 days after induction of arthritis. The tibio-tarsal joints were collected for confirmation of the disease development. The muscles tibialis anterior and gastrocnemius were weighed and processed for the evaluation of myofiber cross-sectional area (CSA) and for the assessment of GDF-8, GDF-11 and GDF-15 gene expression. The CIA group had significantly higher arthritis scores and larger hind paw edema volumes than CO at initial and established disease (25 and 50 days after disease induction). The CIA had decreased grip strength in both time points compared to CO. Sarcoplasmic ratios and muscle weight were also reduced in CIA at established disease. The tibialis anterior CSA was reduced in CIA at established disease compared with the CO (p=0.026). GDF-11 levels were increased in CIA at initial disease and tended to be higher at established disease (p=0.004, p=0.07, respectively). GDF-8 expression was decreased at established disease (p=0.004) and GDF-15 do not differ between groups. A negative correlation between muscle strength and GFD-11 was found at initial disease (r=-71 p=0.071). At initial arthritis, GDF-11 mRNA expression is increased and also associated with loss of grip strength, while GDF-8 gene expression is reduced at established disease, possibly as a compensatory mechanism. Thus, the GDFs may have a role at muscle outcomes in CIA model, and that they can be involved at muscle atrophy and loss of strength.

Keywords: Muscle loss; rheumatoid arthritis; GDF-11; GDF-15; GDF-8.

LISTA DE FIGURAS

LISTA DE ABREVIATURAS

AR	Artrite reumatoide
(HLA)-DRB1	Antígeno leucocitário humano DRB1
ACPA	Anticorpo contra proteínas citrulinadas
RF	Fator reumatoide
Th1	T helper 1
Th17	T helper 17
MMP	Metaloproteinases
RANKL	Receptor ativador do fator nuclear kB
FLS	Fibroblastos sinoviais
IL-1	Interleucina 1
IL-17	Interleucina 17
PCR	Proteína C reativa
GDF	Fator de crescimento e diferenciação
IL-6	Interleucina 6
TNF-ALPHA	Fator de necrose tumoral alfa
MLS	Macrófagos sinoviais
ACR	American College of Rheumatology;
EULAR	Liga Europeia Contra Reumatismo
AINES	Anti-inflamatórios não esteroidais
DMCDs	Drogas modificadoras do curso da doença
MTX	Metotrexato
ICAD	Indices compostos de atividade da doença
IL-1β	Interleucina 1 beta
GDF-15	Fator de crescimento e diferenciação 15
GDF-11	Fator de crescimento e diferenciação 11
GDF-8	Fator de crescimento e diferenciação 8
GDNF	Fator neurotrófico derivado de células glias
TGF-b	Fator de crescimento e diferenciação b

SUMÁRIO

1. INTRODUÇÃO

1.1 Artrite reumatoide

A artrite reumatoide (AR) é uma doença autoimune inflamatória crônica que acomete primordialmente as articulações sinoviais periféricas, porém também possui manifestações extra articulares como nódulos reumatoides, vasculite e perda de massa muscular (SMOLEN e ALETAHA e colab., 2016). A AR é caracterizada por produção de autoanticorpos, sinovite crônica, destruição cartilaginosa e óssea, resultando em incapacidade funcional aos pacientes (KHURANA e BERNEY, 2005). A doença atinge 0.5-1% da população adulta mundial e 0.46% da população brasileira, sendo três vezes mais frequente em mulheres que em homens (SENNA e colab., 2004; TOBÓN e colab., 2010). A incidência de AR aumenta com a idade, atingindo um pico entre os 40 e 50 anos da vida de um indivíduo (SCOTT e colab., 2010). Apesar de não possuir a etiologia totalmente elucidada, sabe-se que a AR tem influência de fatores genéticos e ambientais, os quais afetam sua suscetibilidade e severidade (SENNA e colab., 2004). A hereditariedade da doença é atualmente estimada em 40-65% na AR soropositiva (presença de anticorpos contra auto-antígenos selecionados) e em aproximadamente 20% na doença soronegativa. Além disso, há forte associação entre os alelos do antígeno leucocitário humano (HLA)-DRB, particularmente HLA-DRB1, e a susceptibilidade dos indivíduos. A variação genética no sistema HLA leva a codificação do epítopo compartilhado, o qual corresponde a uma sequência comum de aminoácidos no sulco de ligação ao peptídeo, influenciando na apresentação de antígenos próprios . Outros loci genéticos provavelmente contribuem com efeitos funcionais menores, de maneira isolada ou cumulativa, através de alterações nas vias co-estimulatórias (por exemplo, CD28, CD40), na sinalização de citocinas, no limiar de ativação de receptores linfocitários (por exemplo, PTPN22) e na ativação da imunidade inata (GREGERSEN e colab., 1987; LENZ e colab., 2015; VIATTE e colab., 2015). Os fatores ambientais associados à AR incluem o tabagismo e baixo nível socioeconômico ou escolar como possíveis gatilhos. Alguns indícios também sugerem que agentes infecciosos, como *Porphyromonas gingivalis*, *Proteus mirablis*, *Escherichia coli* e *Epstein-Barr* vírus podem contribuir para o desenvolvimento da doença por meio de mimetismo molecular (EBRINGER e WILSON, 2000).

A patofisiologia da AR é considerada heterogênea, por envolver alteração de sistemas celulares, moleculares e epigenéticos, e tem como consequência comum a quebra da

autotolerância e o estabelecimento da autoimunidade. Nesse contexto, há presença de autoanticorpos (soropositividade), os quais estão associados com danos articulares mais graves e com o aumento da mortalidade (ALETAHA e colab., 2015; GONZALEZ e colab., 2008; HONDA e LITTMAN, 2012; SCHER e colab., 2015; SMOLEN e ALETAHA e colab., 2016; VAN GAALEN e colab., 2004) Os autoanticorpos anti-proteínas citrulinadas (ACPAs) estão presentes em 50-70% dos pacientes com AR e são direcionados a proteínas próprias modificadas, em que resíduos de arginina são substituídos por citrulina (citrulinação). O fator reumatoide (FR) foi o primeiro autoanticorpo descrito na AR e é dirigido contra a porção Fc das imunoglobulinas, o que pode levar à ativação abundante do sistema complemento (ANQUETIL e colab., 2015; SABHARWAL e colab., 1982; ZHAO e colab., 2008). O FR está presente em 50-80% dos indivíduos com AR e, embora ainda importante, apresenta menor especificidade para AR em comparação com o ACPA.

A composição celular da inflamação articular na AR inclui células imunes inatas (monócitos, células dendríticas, mastócitos e células linfóides inatas) e células imunes adaptativas (células Th1 e Th17, células B, plasmoblastos e plasmócitos) que, por sua vez, contribuem para o desenvolvimento de uma resposta autoimune robusta contra os componentes articulares. Além da infiltração celular e da resposta imune exacerbada, na articulação, há presença de citocinas pró inflamatórias como o fator de necrose tumoral alfa (TNF-α), interleucina 1 (IL-1) e interleucina 6 (IL-6), as quais alteram o perfil dos fibroblastos (FLS) e macrófagos sinoviais (MLS) (CHOY, 2012). Dessa forma, os FLS assumem um fenótipo agressivo e resistente à apoptose e são capazes de secretar metaloproteinases de matriz (MMPs), moléculas de adesão e o ligante do receptor ativador do fator nuclear kB (RANKL), promovendo a degradação da cartilagem articular e dano ao osso subcondral (BARRA e colab., 2011; LENZ e colab., 2015).

Em adição, os MLS e FLS que são uma importante fonte de citocinas e proteases, aumentam sua proliferação, levando à hiperplasia da membrana sinovial (FIRESTEIN e MCINNES, 2017). Esta hiperplasia sinovial, juntamente com o infiltrado das células inflamatórias e a estimulação da angiogênese, leva à formação de um tecido invasivo denominado *pannus*, o qual invade as estruturas adjacentes, gerando danos à cartilagem e ao osso, destruindo progressivamente a articulação (FIRESTEIN e MCINNES, 2017).
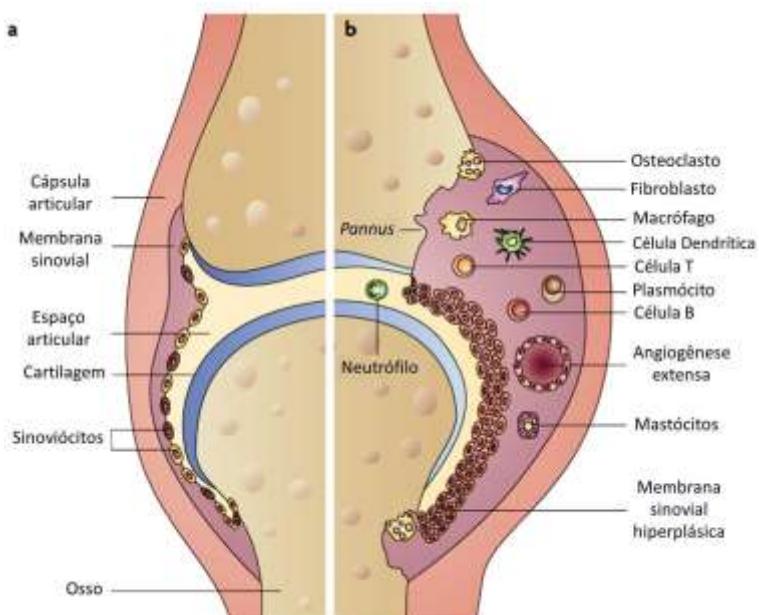
Figura1: Alterações articulares na artrite reumatoide. a) Articulação saudável; b) Articulação doente (Adaptado de Smolen and Steiner, 2003).

Entre as manifestações clínicas apresentadas pelos pacientes com AR estão dor, rigidez, principalmente no período matinal, edema nas articulações, fraqueza, incapacidade funcional (CUTOLO e colab., 2014). Como consequência, diminuição da qualidade de vida é observada nesses pacientes (SMOLEN e ALETAHA e colab., 2016). Além das manifestações já citadas, os pacientes com AR podem apresentar parâmetros clínico-laboratoriais disformes, como a presença de proteína C reativa (PCR) em alta concentração e alterações na velocidade de sedimentação eritrocitária. De acordo com as manifestações clínicas e padrões sanguíneos que os pacientes apresentam, foram criados alguns critérios diagnósticos para classificação de AR pelo Colégio Americano de Reumatologia (*American College of Rheumatology*; ACR) e pela Liga Europeia Contra Reumatismo (*European League Against Rheumatism*; EULAR) (tabela 1) (SINGH e colab., [S.d.]).

Tabela 1. Critérios de classificação para AR segundo ACR 2010 (Aletaha et al., 2010).

1. Envolvimento articular (0-5)

- 1 articulação média a grande (0)
- 2-10 articulações médias a grande (1)
- 1-3 articulações pequenas (não contando articulações grandes) (2)
- 4-10 articulações pequenas (não contando articulações grandes) (3)
- > 10 articulações (pelo menos uma articulação pequena) (5)

2. Sorologia (0-3)

- Fator reumatoide (FR) e Anticorpo contra antígenos citrulinados (ACPA) negativo (0)
- FR e ACPA fracamente positivos (2)
- FR e ACPA fortemente positivos (3)

3. Reagentes de fase aguda (0-1)

- Proteína C reativa e taxa de sedimentação eritrocitária normal (0)
- Proteína C reativa e/ou taxa de sedimentação eritrocitária anormal (1)

4. **Duração dos sintomas (0-1)**

- < 6 semanas (0)
- 6 semanas ou mais (1)

Ponto de corte para artrite reumatoide: 6 ou mais

O tratamento da doença é baseado em uma estratégia que envolve tratar até alcançar o objetivo (*treat-to-target*) e, sempre que necessário, o tratamento deve ser ajustado em avaliações clínicas frequentes (SMOLEN e BREEDVELD e colab., 2016). O tratamento farmacológico visa a remissão ou pelo menos a baixa taxa de atividade da doença, a fim de restaurar a função física na doença precoce e maximizar a função física na doença estabelecida (ABBOTT e MORELAND, 2004; SMOLEN e ALETAHA e colab., 2016). Outros objetivos do tratamento incluem redução da dor, controle de comorbidades extra-articulares e preservação de atividades recreativas e de trabalho (SCOTT e colab., 2010). As terapias medicamentosas incluem uso de anti-inflamatórios não esteroidais (AINEs), corticoides, drogas imunossupressoras e drogas modificadoras do curso da doença (DMCDs) sintéticas e biológicas.

Os AINEs são úteis para diminuir o processo inflamatório e a dor, principalmente no início da doença, pois as DMCDs não têm ação imediata, e podem ser empregados quando não se obtém controle completo da atividade e em reagudizações da AR. O efeito mais conhecido e esperado dos corticoides na AR é a melhora do processo inflamatório e da dor, contudo, atualmente são indicados na politerapia em associação com as DMCDs. A base do uso de imunossupressores para o tratamento da AR inclui redução da resposta celular e propriedades anti-inflamatórias (interferência sobre a migração e a ação de neutrófilos, linfócitos e monócitos) na sinovite e em outras manifestações extra-articulares da doença.

As DMCDs devem ser indicadas ao paciente a partir da definição do diagnóstico de AR. O metotrexato (MTX) é um agente imunomodulador cuja ação consiste na inibição da síntese de DNA, RNA, timidinato e proteínas. O MTX é considerado o fármaco padrão no

tratamento da AR e apresenta capacidade de reduzir sinais e sintomas de atividade da AR, além de reduzir a progressão das lesões radiográficas. Um dos mais relevantes avanços na terapia da AR foi o desenvolvimento das DMCDs biológicas (M. e colab., 2016). As DMCDs biológicas estão indicadas para os pacientes que persistam com atividade da doença, apesar do tratamento com pelo menos dois esquemas de DMCDs sintéticas. Encontram-se aprovadas para uso no Brasil as seguintes DMCDs biológicas: *a)* anti-TNF: adalimumabe, certolizumabe, etanercepte, infliximabe e golimumabe; *b)* depletor de linfócito B: rituximabe; *c)* bloqueador da co-estimulação do linfócito T: abatacepte; *d)* bloqueador do receptor de interleucina-6 (IL-6): tocilizumabe. Há também uma nova classe de medicamentos chamados DMARDs sintéticos alvo-específicos, como o tofacitinibe utilizado para pacientes com AR ativa que tenham apresentado falha terapêutica a DMCDs sintéticas ou aos agentes inibidores do TNF (TNFi) (MOTA e colab., 2015; SMOLEN e colab., 2017).
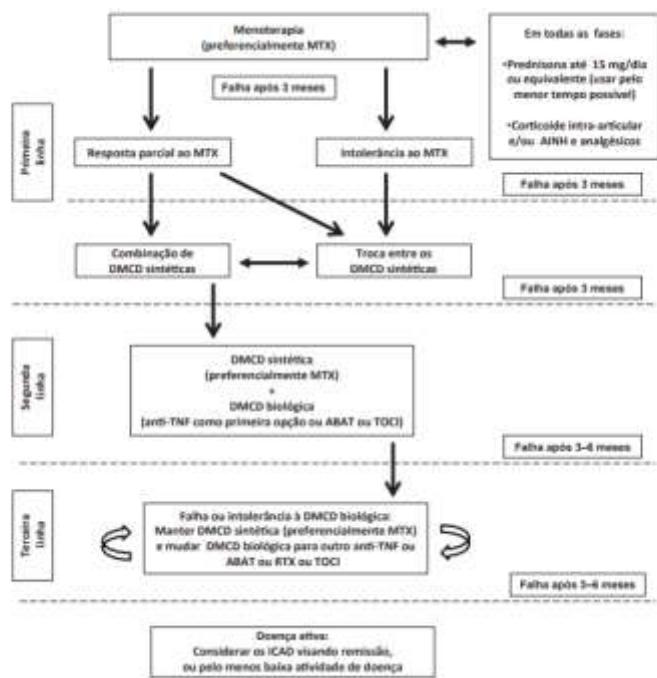
Figura 2. Fluxograma de tratamento medicamentoso para a AR da Sociedade Brasileira de Reumatologia no Brasil, proposto pela Comissão de AR da SBR. DMCD, drogas modificadoras do curso da doença; MTX, metotrexato; anti-TNF, medicações antifator de necrose tumoral; ABAT, abatacepte; RTX, rituximabe; TOCI, tocilizumabe; ICAD, índices compostos de atividade da doença.

Um outro aspecto importante no tratamento da AR, é o manejo das comorbidades associadas, como doenças cardíacas, renais e depressão, pois elas refletem tanto o processo

da doença como o seu tratamento (SCOTT e colab., 2010). Apesar dos avanços no tratamento da AR e das diversas classes de medicamentos disponíveis, os tratamentos farmacológicos, por vezes, não são capazes de atenuar danos extrarticulares como déficits musculoesqueléticos, os quais resultam em declínio da função física e da qualidade de vida dos pacientes.

1.2 Envolvimento muscular na artrite reumatoide

Além do acometimento articular, estudos retratam a AR como uma doença com características extra articulares importantes associadas com a intensidade da sinovite e com o aumento da atividade da doença (NYHÄLL-WÅHLIN e colab., 2009). Por outro lado, mesmo com o controle da atividade da doença, a prevalência de prejuízos na função física entre pacientes com AR é alta, em comparação com indivíduos controles (LEMMEY e colab., 2016). Frequentemente, esses pacientes apresentam achados como fadiga, fraqueza e atrofia generalizada de fibras musculares (YOUNG e KODURI, 2007).

A perda muscular afeta de 10-67% dos pacientes (ROUBENOFF e colab., 1994) e a baixa massa magra, presente em cerca de 20% dos pacientes com AR, está relacionada com a redução da atividade física, com o aumento da fadiga e com a perda de força (VAN BOKHORST-DE VAN DER SCHUEREN e colab., 2012). Consequentemente, esse quadro leva a uma perda significativa da capacidade funcional e da qualidade de vida, e está relacionado com um impacto econômico elevado (BURNHAM e RUSSELL, 1986; FLEMING e colab., 1976; MUNRO e CAPELL, 1997). Já foi demonstrado que na AR inicial há efeito da doença na função física e esta também sofre efeito das comorbidades aumentando o risco de mortalidade. Além disso, demonstrou-se que indivíduos com AR apresentam déficits mais pronunciados na área e na densidade muscular quando há baixa massa gorda, e que o aumento da destruição articular está associado a maiores déficits musculares (BAKER e colab., 2014). Entretanto, poucas medidas são tomadas para remediar a perda de função física e muscular nos pacientes com AR e, dessa forma, melhorar a qualidade de vida; alguns dados apresentam o exercício físico como melhoria do desempenho físico, a aptidão cardiorrespiratória e da força muscular, além de reduzir a atividade da doença e a inflamação sistêmica, e, portanto, um modo a melhorar a capacidade funcional dos pacientes (LUNDBERG e NADER, 2008).

Uma série de fatores estão associados com a perda muscular na AR, como exposição crônica a citocinas pró-inflamatórias, principalmente TNF-α, IL-1β e IL-6, alterações

hormonais e inatividade física, além de ingestão inadequada de proteínas e tratamento com glicocorticoides – todos resultando em redução de síntese e aumento de degradação de proteínas musculares (SAKUMA e YAMAGUCHI, 2012; TRACEY e colab., 1990; TURESSON e colab., 2002). Em um estudo envolvendo indivíduos com AR, os níveis de citocinas musculares não refletem os níveis sistêmicos de citocinas, mas foram duas vezes maiores no tecido muscular. Esse achado sugere que, na AR, as citocinas musculares podem ser produzidas localmente por miofibras, células inflamatórias residentes e/ou adipócitos (HUFFMAN e colab., 2017). Além disso, a intensidade da inflamação e a severidade da doença estão associadas com a perda muscular na AR (FUKUDA e colab., 2010). A alta severidade da AR resulta em aumento dos mediadores inflamatórios e dessa forma, no aumento da inflamação sistêmica. A inflamação é um importante contribuinte para a disfunção do músculo esquelético e há indícios de que citocinas pró-inflamatórias como o TNF-α e a IL-1 provavelmente atuam como mediadores centrais da perda de massa muscular na AR (JACKMAN e KANDARIAN, 2004); estudos em murinos mostram que o bloqueio do TNF-α resgata a perda de músculo esquelético, sugerindo que o TNF-α funciona como um importante contribuinte da perda muscular. Por outro lado, o TNF-α pode não ser o único mediador, visto que a inibição concomitante de IL-1 e TNF-α é mais eficaz na redução do declínio muscular (ROUBENOFF e colab., 1997).
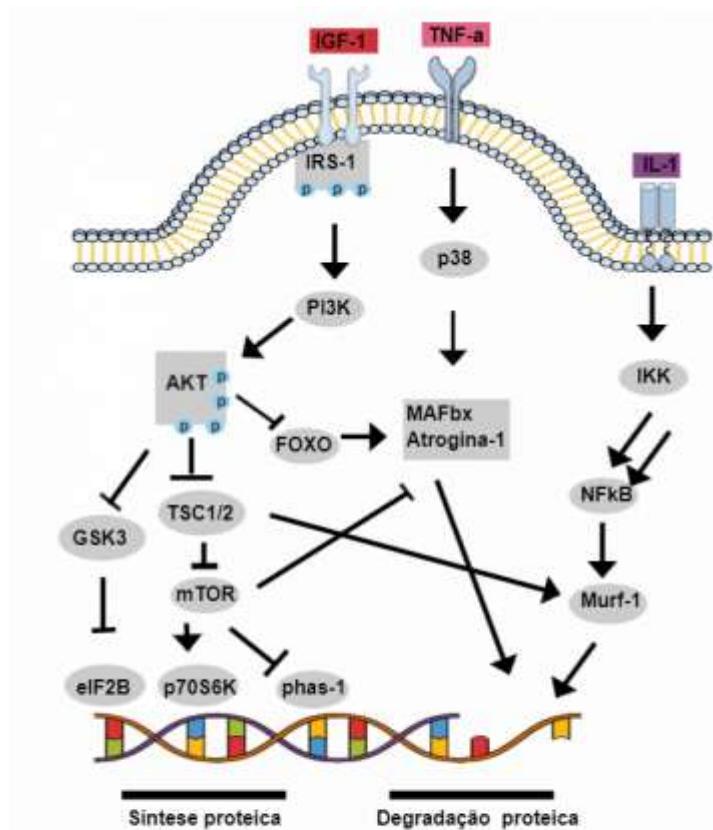
Figura 3. Vias de sinalização de perda muscular. (Elaborada pelo autor)

Os efeitos debilitantes da perda de massa muscular reduzem a qualidade de vida e a sobrevivência dos pacientes com AR, no entanto, há pouco conhecimento a respeito de vias intracelulares e de proteínas envolvidas nesse processo da doença. Assim, é necessário entender os mecanismos que regulam os déficits musculares para avançar no desenvolvimento de terapias específicas.

1.3 Fatores de crescimento e diferenciação

A superfamília do fator de crescimento transformador beta (TGF-β) compreende um grande número de proteínas secretadas, entre elas os fatores de crescimento e diferenciação (GDF)-8, GDF-11 e GDF-15 (o qual, posteriormente, foi classificado como pertencente ao fatores neurotróficos derivados de células glias (GDNF). Os GDFs regulam vários processos

biológicos fundamentais como o desenvolvimento embrionário e a regulação pós-natal de órgãos (SARTORI e colab., 2014b). GDF-8 e GDF-11 realizam a transdução de sinal pelos receptores activina tipo IIB e tipo IIA (ActRIIB/IIA) e, sequencialmente, ativam fatores de transcrição Smad 2 e 3 (AMTHOR e colab., 2009; MASSAGUÉ, 2012; MASSAGUÉ e colab., 2005). Alguns estudos descrevem a sinalização via Smads 2 e 3 como um regulador negativo do crescimento muscular podendo induzir um quadro de atrofia intensa (AMTHOR e colab., 2009; MASSAGUÉ, 2012; MASSAGUÉ e colab., 2005). Em vista disso, ligantes TGF-β podem desempenhar um papel relevante na homeostase entre a degradação e a síntese proteica e contribuir para a fisiologia muscular.

O GDF-8 (também conhecido como miostatina) é um regulador negativo do crescimento muscular esquelético, principalmente por meio da diminuição da miogênese (SHARMA e colab., 2001). Estudos com animais nocaute, ou com mutação no gene do GDF-8, demonstraram aumento da massa muscular pelos processos de hipertrofia e hiperplasia (GROBET e colab., 2003). Em modelos animais de AR, já foi reportado que não há alteração na expressão proteica de miostatina no músculo (DE OLIVEIRA NUNES TEIXEIRA e colab., 2013). Por outro lado, outro estudo demonstrou que a expressão proteica de miostatina está diminuída tanto no músculo quanto no soro de animais artríticos (LITTLE e colab., 2017). Em estado de remissão, foi observada uma diminuição da expressão de miostatina, possivelmente por conta do efeito positivo da terapia anti-inflamatória em mediadores que influenciam a miogênese (KERSCHAN-SCHINDL e colab., 2019).

Nas membranas sinoviais de pacientes, mais especificamente nos FLS, a miostatina pode estar estimulando a produção de IL-1β, via inibição de miR-21-5p, um regulador negativo da produção de IL-1β, como também é capaz de induzir TNF-⟨ via PI3K-AKT (HU e colab., 2017; SU e colab., 2019). Em adição, há aumento significativo na expressão articular de miostatina, em comparação com pacientes com osteoartrite (OA), e esse aumento é capaz de estimular a osteoclastogênese e contribuir para a degradação óssea da doença (LU e colab., 2016).

O GDF-11 apresenta um estreito parentesco com o GDF-8, uma vez que possuem cerca de 90% de identidade conformacional. Por conta disso, sugere-se que o GDF-11 também pode ser capaz de regular negativamente o crescimento da massa muscular (NAKASHIMA e colab., 1999). Além disso, embora a sinalização de GDF-8 e de GDF-11 ocorra pelo mesmo receptor, demonstrou-se que o GDF-11 é um ligante mais potente para a ativação da via ActRII/Alk/Smad 2/3 do que o GDF-8, uma impressão que foi confirmada,

posteriormente, pela análise da estrutura proteica (EGERMAN e colab., 2015; NAKASHIMA e colab., 1999; TRENDELENBURG e colab., 2009). Com relação à ação do GDF-11, alguns estudos avaliaram a sua expressão e influência sobre a musculatura esquelética ao longo do envelhecimento. Inicialmente, demonstrou-se em camundongos que os níveis sanguíneos de GDF-11 diminuem com o envelhecimento, e que a administração de GDF-11 é capaz de promover a reversão do declínio muscular esquelético relacionado à idade, bem como a reversão da hipertrofia cardíaca (LU e colab., 2016; POGGIOLI e colab., 2016; SINHA e colab., 2014). Por outro lado, também foi reportado que os níveis séricos de GDF-11 aumentam com a idade em camundongos e humanos e que a administração de GDF-11 em camundongos provoca diminuição da regeneração muscular (EGERMAN e colab., 2015). Por fim, um estudo demonstrou que em homens saudáveis os níveis circulantes de GDF-11 não diminuem com o envelhecimento, mas, ao invés disso, os níveis de miostatina são menores em homens idosos em comparação com homens mais jovens (SCHAFER e colab., 2016). Em modelo de AR foi observado que o GDF-11 antagoniza a inflamação induzida por TNF e protege contra o desenvolvimento de artrite inflamatória em camundongos (LI e colab., 2019).

Além dos efeitos sobre o músculo, há evidências de que o GDF-11 também é capaz de induzir a perda de apetite através de um mecanismo indireto, no qual as altas concentrações musculares de GDF-11 provocam uma elevação plasmática de GDF-15 (EMMERSON e colab., 2017; JONES e colab., 2018; MULLICAN e colab., 2017). Por sua vez, o GDF-15, é capaz de ativar, diretamente, neurônios hipotalâmicos e, dessa maneira, levar à perda de apetite e, eventualmente, à anorexia (JOHNEN e colab., 2007). Em condições fisiológicas, o GDF-15 é produzido em níveis baixos, mas fatores como lesão ou malignidade podem induzir aumento na sua expressão (JONES e colab., 2018).

Juntas, as proteínas TGF-β e seus componentes sinalizadores exercem controle fisiológico sobre a proliferação, diferenciação, apoptose, adesão e deposição de matriz extracelular, controlando assim a embriogênese, organogênese e homeostase do tecido adulto. Além disso, há evidências crescentes de que as proteínas TGF-β agem em conjunto para regular o crescimento e o remodelamento do músculo esquelético (MASSAGUÉ, 2012; MASSAGUÉ e colab., 2005; SARTORI e colab., 2014a). Cada vez mais a perda de massa muscular observada na doença crônica está sendo associada à regulação perturbada da rede de sinalização do TGF-β e, por conta disso, torna-se importante a ampliação do conhecimento sobre os níveis de GDFs na AR.

1.4 JUSTIFICATIVA

Diante da base teórica apresentada, sabe-se que a perda muscular é prevalente e que afeta profundamente a funcionalidade e a qualidade de vida dos pacientes com AR. Estudos recentes têm ressaltado a importância da família TGF-β no acometimento muscular decorrente do envelhecimento. Devido à escassez de informações sobre o tema na AR, a investigação de possíveis fatores que desencadeiam a perda muscular poderá auxiliar no esclarecimento da fisiopatogenia da doença, bem como na busca por novos alvos terapêuticos. Portanto, a avaliação dos níveis de GDFs no modelo de artrite induzida por colágeno (CIA) é uma boa ferramenta para o entendimento dos mecanismos envolvidos na perda muscular da AR.

1.5 OBJETIVOS

1.5.1 Objetivo geral

Avaliar os níveis séricos e musculares de GDF-8, GDF-11 e GDF-15 ao longo do desenvolvimento da artrite induzida por colágeno (CIA).

1.5.2 Objetivos específicos

- Avaliar o peso corporal;

- Avaliar o escore clínico da doença;

- Avaliar o edema dos membros pélvicos;

- Avaliar a força muscular;

- Medir a área transversal da miofibra;

- Avaliar a transcrição gênica de GDF-11, GDF-8 e GDF-15 no músculo esquelético por PCR;

2 ARTIGO CIENTÍFICO

O presente trabalho foi escrito em forma de artigo científico de acordo com as normas de publicação da revista intitulada - Journal of Cachexia, Sarcopenia and Muscle

The role of grow differentiate factors 8, 11, 15 on muscle wasting in collagen induced arthritis model

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Abstract

Background: Rheumatoid arthritis (RA) is an autoimmune, inflammatory disease which affects primarily synovial joints and can lead patients to muscle deficits. It is known that growth differentiation factor (GDF)-8 11 and 15 play an important role in muscle homeostasis. Thus the aim of this study was to evaluate muscle mRNA expression of GDF-8, GDF-11 and GDF-15 throughout the development of collagen-induced arthritis (CIA) and its associations with clinical parameters.

Methods: Male DBA/1J mice, between 8 and 12 weeks of age, were randomly divided into three experimental groups: healthy animals (HC, n=6), control animals without intervention (CO, n=16) and collagen-induced arthritis animals (CIA, n=16). During the experimental period, disease score and edema, and grip strength were evaluated. Mice were euthanized at day 0. 25 or 50 days after induction of arthritis. The tibio-tarsal joints were collected for confirmation of the disease development. The muscles tibialis anterior and gastrocnemius were weighed and processed for the evaluation of myofiber cross-sectional area (CSA) and for the assessment of GDF-8, GDF-11 and GDF-15 gene expression.

Results: The CIA group had significantly higher arthritis scores and larger hind paw edema volumes than CO at initial and established disease (25 and 50 days after disease induction). The CIA had decreased grip strength in both time points compared to CO. Sarcoplasmic ratios and muscle weight were also reduced in CIA at established disease. The tibialis anterior CSA was reduced in CIA at established disease compared with the CO (p=0.026). GDF-11 levels were increased in CIA at initial disease and tended to be higher at established disease (p=0.004, p=0.07, respectively). GDF-8 expression was decreased at established disease (p=0.004) and GDF-15 do not differ between groups. A negative correlation between muscle strength and GFD-11 was found at initial disease (r=-71 p=0.071).

Conclusions: At initial arthritis, GDF-11 mRNA expression is increased and also associated with loss of grip strength, while GDF-8 gene expression is reduced at established disease, possibly as a compensatory mechanism. Thus, the GDFs may have a role at muscle outcomes in CIA model, and that they can be involved at muscle atrophy and loss of strength.

Keywords: Muscle loss; rheumatoid arthritis; GDF-8; GDF-11; GDF-15.

Background

Rheumatoid arthritis (RA) is a chronic, autoimmune, debilitating disease that generally occurs within the fourth and sixth decade of life and affects more commonly women than men[1]. RA is primarily characterized by joint pain, swelling, stiffness, and about 40% of patients present extra-articular manifestations, such as muscle deficits, either in the beginning or during the course of the disease[2–6]. RA predisposes to changes on body composition, which lead to decreased lean mass and increased fat mass, reducing health-related quality of life and increasing mortality [3,7]. It has been reported that low muscle mass is present in 20% and 38% of the patients with initial and established disease, respectively [8–11]. In RA, muscle wasting is also associated with weight loss, decreased physical activity, as well as increased fatigue and weakness, all of which can further compromise functional capacity[7,12]. Additionally, low appendicular lean muscle mass and low thigh muscle density were associated with various disability measures leading patients to frailty [11,13,14].

It is widely believed that pro-inflammatory cytokines, including TNFα, IL-1β, IL-6, and IFN-γ, play an important role in the pathogenesis of RA, as they are involved in the development of synovitis and extra-articular manifestations of the disease[15–17]. Furthermore, TNF-α and IL-1 are also involved as central mediators of muscle mass loss in RA[18]; murine studies show that TNF-α blockade rescues the loss of skeletal muscle, suggesting that TNF-α acts as a major contributor to muscle loss, although it is probably not the only mediator [19,20]. However, the triggers that can lead to muscle loss in RA are not fully elucidated.

TGF-β cytokines family has been associated with the fibrosis seen in older tissue and as inhibitors of muscle differentiation in aging and frailty[21–23]. Growth differentiation factor (GDF)-8, act as an inhibitor of muscle differentiation and induce atrophy on post-differentiated myotubes[24,25]. The increase in muscularity upon the loss of GDF-8 has been demonstrated in multiple animals and even in humans[26,27]. In experimental arthritis, protein expression of GDF-8 was decreased in both muscle tissue and serum of arthritic animals [28]. Also, in RA patients in remission, decreased serum GDF-8 was observed, possibly due to the positive effect of anti-inflammatory therapy on mediators that influence the myogenesis[20]. In other TGF-β family molecules, distinct from GDF-8, a role in modulating skeletal muscle size have been observed, since GDF-8 knockout mice that are mated with mice that are transgenic for follistatin, which is capable of inhibiting not only GDF-8 but also its close relative GDF-11, resulted in an even greater increase in muscle size [29–31]. GDF-11 play critical roles in embryonic development, skeletal metabolism, and muscle formation and shares with GDF-8 90% of structural similarity [27,30]. Besides the high similarity, recent

manuscripts reported that, in mice, GDF-11 is able to decrease cardiac-related muscle hypertrophy and that GDF-11 levels decrease with aging[32]. Later, in a distinct study, it was shown that GDF-11 has positive effects on aged satellite cells (SCs) and that the administration of GDF-11 to older mice improves skeletal muscle regeneration[33]. In addition to the effects on muscle, GDF-11 is also able to induce loss of appetite through an indirect mechanism, since the increased muscle GDF-11 promotes changes in GDF-15 plasma levels [18,34–36]. As consequence, GDF-15 is capable of directly activating hypothalamic neurons, thereby causing loss of appetite and eventually anorexia. Despite the knowledge about TFG-β cytokines family, the role of GDF-8, GDF-11 and GDF-15 remains doubtless and not fully understood in RA pathogenesis. Therefore, given the role of these molecules on skeletal muscle, it seemed important to study the role of GDFs, *in vivo,* in RA model.

Material and Methods

Animals: Male DBA/1J mice, between 8 and 12 weeks of age, were randomly divided into five experimental groups: healthy animals (HC=6); which were used as basal qPCR expression analysis of target genes, control animals without intervention (CO, n=8) ;euthanized after 25 days of disease, collagen-induced arthritis animals (CIA, n=8); euthanized after 25 days of diasese to evaluated a initial CIA, control animal euthanized ater 50 days of disease and CIA established (CIA, n=8). To evaluate GDFs expression and clinical parameters, mice were euthanized at day 0. 25 or 50 days after induction of arthritis. The mice were reared at 20°C, with a 12-h light–dark cycle, food and water were provided *ad libitum*. Animals were followed up for 50 days, and all measurements were performed prior to the arthritis induction and 0. 18, 25, 50 days thereafter. All experiments were performed following to the Guiding Principles for Research Involving Animals. This study was approved by the Research Ethics Committee of the Hospital de Clínicas de Porto Alegre (protocol no. 180367). Arthritis was induced with bovine type II collagen (CII; Chondrex, Inc., Redmond,WA, USA; 2 g·mL1) dissolved in 0.1M of acetic acid at 4°C for 12 hours and in Complete Freund's Adjuvant (CFA; Sigma, St Louis, USA; 2 mg·mL1) containing inactivated *Mycobacterium tuberculosis*[37]. Fifty microliters of emulsion (CII + CFA) were injected intradermally at the base of the tail to induce arthritis; this was set as the day zero in this experiment. At this point, HA animals were euthanized for posterior analysis. Eighteen days after the first injection, the animals received a reinforcement of CII emulsified with incomplete Freund's adjuvant (without *M. tuberculosis*) in another site of the tail (*booster*

injection)[38]. During the procedures, mice were anaesthetized with isoflurane 10% (Abbott Laboratórios do Brasil Ltda., Brazil) and 90% of oxygen. Healthy controls were manipulated and anaesthetized; however, no injection was made. Animals were euthanized at 25 or 50 days after the first injection. To the following analysis as a randomized study, a group of researchers was blinded until animals developed signs of the disease. In addition, all histological and molecular analyses were performed by blinded researchers.

Clinical severity score and measurement of edema: Arthritis severity was clinically determined for each paw, three times a week, according to the a scale of 0 to 4 (0. no evidence of erythema and swelling; 1, erythema and mild swelling confined to the tarsals or metatarsals; 2, erythema and moderate swelling of tarsal and the metatarsal or tarsal and ankle joints; 3, erythema and severe swelling extending from the ankle to metatarsal joints; and 4, erythema and severe swelling encompassing the ankle, foot and digits, or ankylosis of the limb). The highest sum score that a mouse could reach was 16. Hind paw edema volume was measured using a plethysmometer (Insight Ltda., Ribeirão Preto, Brazil). Briefly, it is a small cylinder filled with a buffer connected to a device capable to measure the total fluid volume, we had immersed the hind pawn of the animal inside the cylinder and the total volume added is then measured, the difference between the final volume minus the initial volume results to pawn total volume.

Grip strength: Animals were tested for maximum grip strength with a test adapted from Deacon et all.[39]. Briefly, first, we used meshes with proper loads, each one amounting 5, 20. 35, 50. 65, 80. and 95 grams. Each mouse was held by the first third of the tail and suspended until it grasped the lighter weight with all paws. The animal had to hold the load for at least 3 recorded seconds. If the animal succeeded, it rested for 30 seconds before trying the next weight. If the animal failed three times with a 10-seconds rest between each attempt, the longest time it was able to hold the weight was recorded. The following equation was used: Fmax = P3seg + (5*t<3seg), where Fmax is the maximum calculated grip strength, P3seg is the heaviest load the animal held for 3 s, and t<3seg is the longest time the animal held the heaviest load. The final result was expressed in grams (g).

Organs and tissue dissection: At days 0. 25 and 50 the mice were euthanized and the muscles tibialis-anterior and gastrocnemius were collected for histological analysis. The gastrocnemius muscle from the other paw was dissected immediately after euthanasia,

weighed and frozen at -80°C for posterior gene expression analysis. The tibio-tarsal joints were collected to confirm the development of arthritis by histopathological analysis.

Histological analysis: The tibio-tarsal joint, tibialis anterior, and gastrocnemius muscle of the DBA/1J animals were dissected and immersed in 10% buffered formalin for fixation for up to 3 days. Next, the tibio-tarsal joints were decalcified in 10% nitric acid for 24 hours. All these tissues were dehydrated and embedded in paraffin blocks. Slices 6-μm thick were arranged on microscope slides. We used a histological score system to evaluate individual joints and assess arthritis severity. For synovial inflammation, five high-power magnification fields were scored for the percentage of infiltrating mononuclear cells as follows: 0. absent; 1, mild (1–10%); 2, moderate (11–50%); 3, severe (51–100%); for synovial hyperplasia: 0. absent; 1, mild (5–10 layers); 2, moderate (11–50 layers); 3, severe (>20 layers); for extension of pannus formation based on the reader's impression: 0. absent; 1, mild; 2, moderate; 3, severe; for synovial fibrosis: 0. absent; 1, mild (1–10%); 2, moderate (11–50%); 3, severe (51–100%); for cartilage erosion, that is, the percentage of the cartilage surface that was eroded: 0. absent; 1, mild (1–10%); 2, moderate (11–50%); 3, severe (51–100%); and for bone erosion: 0. none; 1, minor erosion(s) observed only at high-power magnification fields; 2, moderate erosion(s) observed at low magnification; 3, severe transcortical erosion(s).

Animal weight and muscle weight: Animals were weighed for total body mass three times a week starting before the first injection. At 25 and 50 days after the induction of the disease, the tibialis anterior and was dissected immediately after euthanasia, weighed and collected to measure myofiber cross sectional area by histological analysis with haematoxylin-eosin (HE) staining.

Muscle fiber cross-sectional area (CSA): Tibialis anterior stained with HE were used for myofiber diameter measurement. One transverse section of each muscle was stained with HE and analyzed under an optic microscope (×400). Two straight lines crossing at a right angle at the fiber center were drawn in each myofiber. The mean of these diameters (in micrometers) was used to calculate the transverse section mean, based on circle area. For measuring the myofiber diameter of the whole muscle, we took 10 pictures of each section, and 20 fibers were measured from each picture using the Image-Pro Express software (version 5.1.0.12, Media Cybernetics, Rockville, MD, USA).

qPCR analysis: RNA from mouse gastrocnemius muscle was isolated using a RNEasy Mini Kit (Qiagen), per the manufacturer's protocol and the integrity of mRNA was evaluated through 260/280 ratio quantified at nanodrop (Thermo Fisher); If ratio was near 2, the mRNA extraction was considered satisfying. The cDNA synthesis was performed using a High Capacity cDNA Reverse Transcription kit (Applied Biosystems) and used 1µg of mRNA. The cDNA was then used for quantitative PCR (qPCR) using an QuantStudio 5 system and software in combination with Taqman® fast PCR Master Mix (Applied Biosystems). The Taqman probes for GDF-8 (Rn00569683_m1), GDF-11 (Rn01756258_m1) and GDF-15 (Mm00442228_m) were purchased from Applied Biosystems. The PCR conditions consisted in one cycle of denaturation at 95ºC for 20 seconds and 40 cycles of amplification consisting of a denaturation step at 95ºC for 1 second and annealing/elongation step at 60ºC for 20 seconds. Transcript levels were normalized to a reference gene GAPDH according to a previously study that investigated GDF family in muscle. The fold change relative to samples was calculated as $2-\Delta CT$.

Statistical Analysis: Sample size was based on the previous research of our group, in which the main outcome was muscle atrophy accessed by myofiber cross-sectional area (38). The data did not have a Gaussian distribution by Shapiro–Wilk and Kolmogorov–Smirnov tests, so quantitative data is described as medians and interquartile range. For mRNA expressions and histopathological analysis, comparison between CIA and CO groups was performed using Mann Whitney *U* test, and comparison between all groups was performed using Kruskall Wallis test. Correlations were determined using Spearman's correlation test. Myofiber and muscle weight comparisons between CO and CIA were performed using one-way ANOVA followed by Tukey's test; Grip strength, hind paw edema an disease score analysis were performed using two-way ANOVA followed by Bonferroni's test; data is presented as mean ± standard error of the mean (SEM). All statistical tests were performed in Statistical Package for the Social Science software, version 18. Statistical difference was assumed for a p value under 0.05.

Results

Arthritis score, edema, and arthritis histopathology: Incidence of arthritis was 100% at 25 days after disease induction (figure 1A). CIA animals had significantly higher arthritis scores and hind paw edema volumes than CO in both initial and established disease (figure 1B). The

histopathology analysis showed that all control animals showed healthy tibio-tarsal joints (Figure 1C), while it confirmed the disease in all CIA animals (Figure 1D). Histopathology parameters of tibio-tarsal joints of CO and CIA groups are showed at **Table 1.**

Figure 1 Follow up of experimental arthritis development. **(A)** Arthritis score and **(B)** hind paw edema volume of CO and CIA groups during the experimental period. Representative histopathology of ankle joint in **(C)** CIA and **(D)** CO groups at days and 50 after disease induction. Legend: A, angiogenesis; B, bone; BE, bone erosion; C, cartilage; CE, cartilage erosion; P, invasive pannus formation; S, synovial layer; and SH, synovial hyperplasia. Data are presented as mean ± standard error of the mean (SEM) (**Table1**). Statistical analysis between groups was performed using two-way analysis of variance followed by Bonferroni's test. $*P < 0.05$
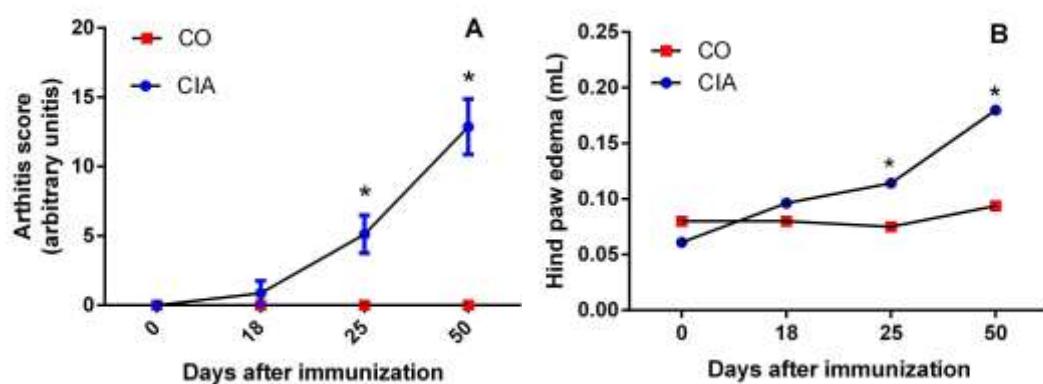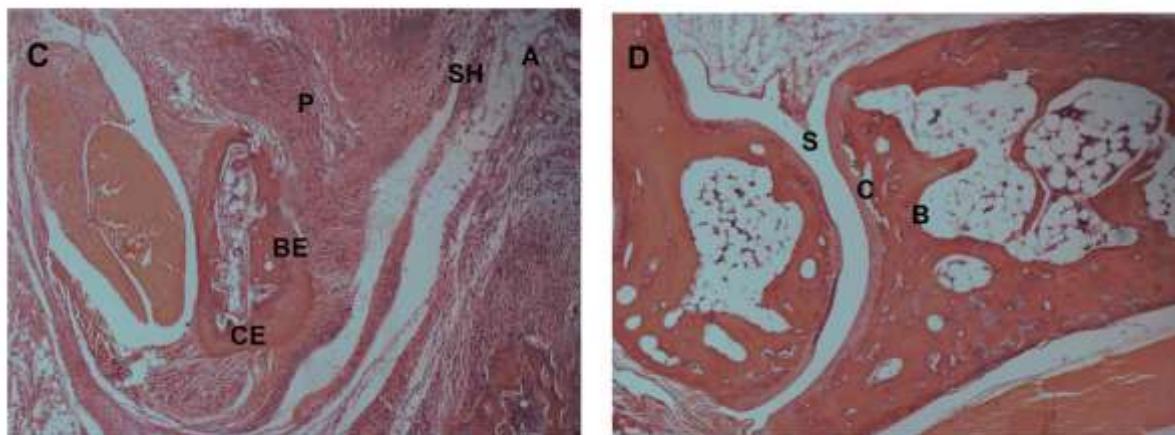
Table 1 Histopathology parameters of tibio-tarsal joints of CO and CIA groups. Cartilage and bone erosion, synovial hyperplasia, invasive *pannus* formation, and inflammatory cells infiltrates were measured. Statistical analysis between groups was performed using Mann Whitney *U* test and results are shown as median and interquartile range.

	CIA	CO
Inflammatory infiltration	3 (2,3)*	0 (0,0)
Synovial hyperplasia	2 (2,3)*	0 (0,1)
Pannus Extension	3 (2,3)*	0 (0,0)
Cartilage erosion	3 (2,3)*	0 (0,0)
Bone erosion	2 (2,2)*	0 (0,0)
Synovial fibrosis	2 (2,3)*	0 (0,0)

*p<0.05

Grip strength assessment: In comparison with CO group, CIA group presented significantly decreased grip strength in both initial (p=0.0072) and established (p=0.0011) disease (Figure 2).

Figure 2 Grip strength of CO and CIA groups during the experimental period. Data are presented as mean ± standard error of the mean (SEM). Statistical analysis between groups was performed using two-way ANOVA followed by Bonferroni's test. *P < 0.05.
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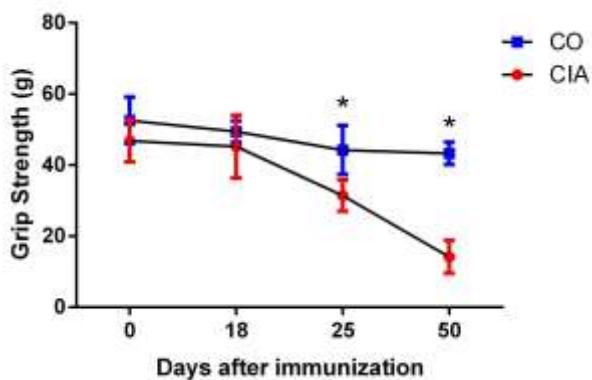
Muscle weight and sarcoplasmic ratio: The dissected tibialis anterior and gastrocnemius muscles weighed less in CIA group (Figure 4A), compared to CO group (Figure 4B), in established disease (p<0.05; Figure 3A, 3B, respectively). Sarcoplasmic ratios (muscle weight in milligrams divided by animal body weight in grams) were also lower in CIA group (Figure 4C), compared to CO group (Figure 4D), in established arthritis (P < 0.05).

Figure 4 (A) and **(B)** Tibialis anterior and gastrocnemius muscle weights and in CIA and CO groups at the end of the experimental period. **(C)** and **(D)** Sarcoplasmic ratio in CIA and CO groups at the end of the experimental period. Data are presented as mean ± standard error of the mean (SEM). Statistical analysis between groups was performed using one-way ANOVA followed by Tukey's test. *p < 0.05, **p<0.01 ***p<0.001
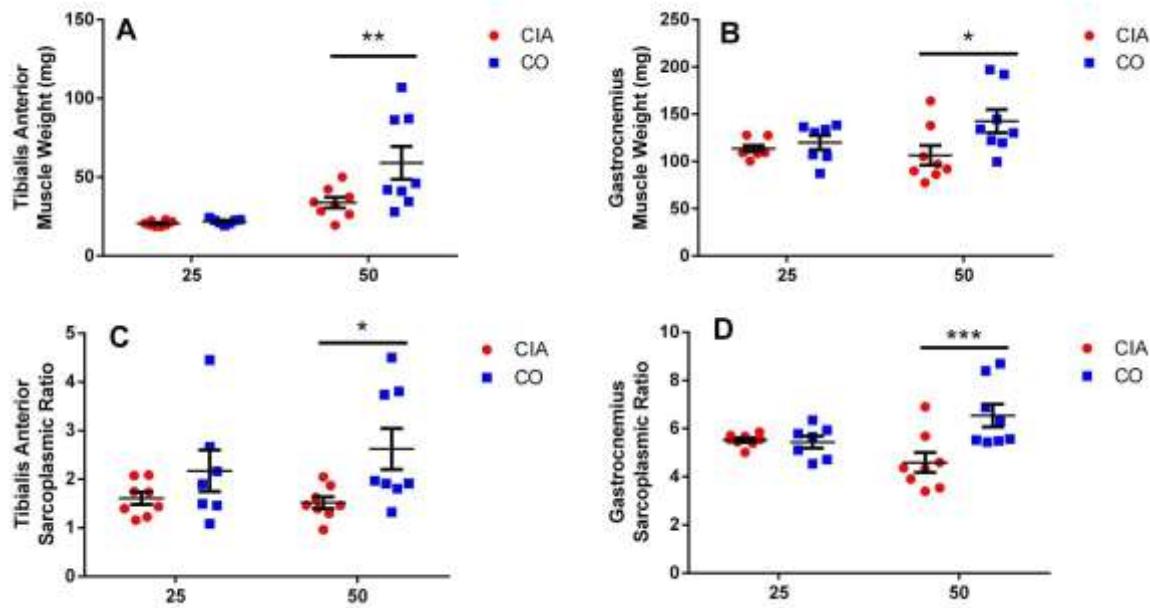
Myofiber cross-sectional area: At day 25, initial disease, there was no difference in tibialis anterior myofiber CSA among the experimental groups. The myofiber CSA of CIA group was significantly lower than CO group at established disease (CIA: 419.9±13.7 mm vs CO: 455.5±8.9 mm; p=0.026; Figure 5A).

Figure 5 (A) Myofiber cross-sectional area of the tibialis anterior muscle of CO and CIA groups at days 25 and 50. Representative histology of tibialis anterior muscle of CIA **(B, D)** and CO **(C, E)** mice. Data are presented as mean ± standard error of the mean (SEM). Statistical analysis between groups was performed using one-way ANOVA followed by Tukey's test. *P < 0.05.
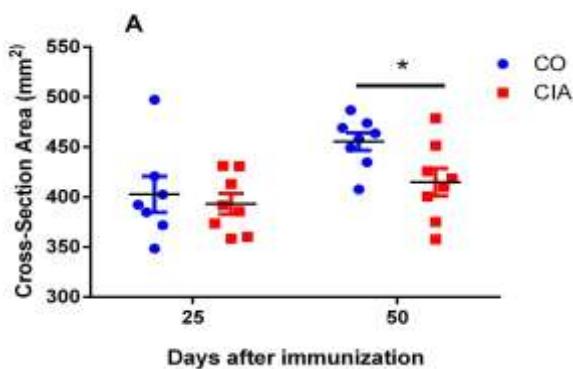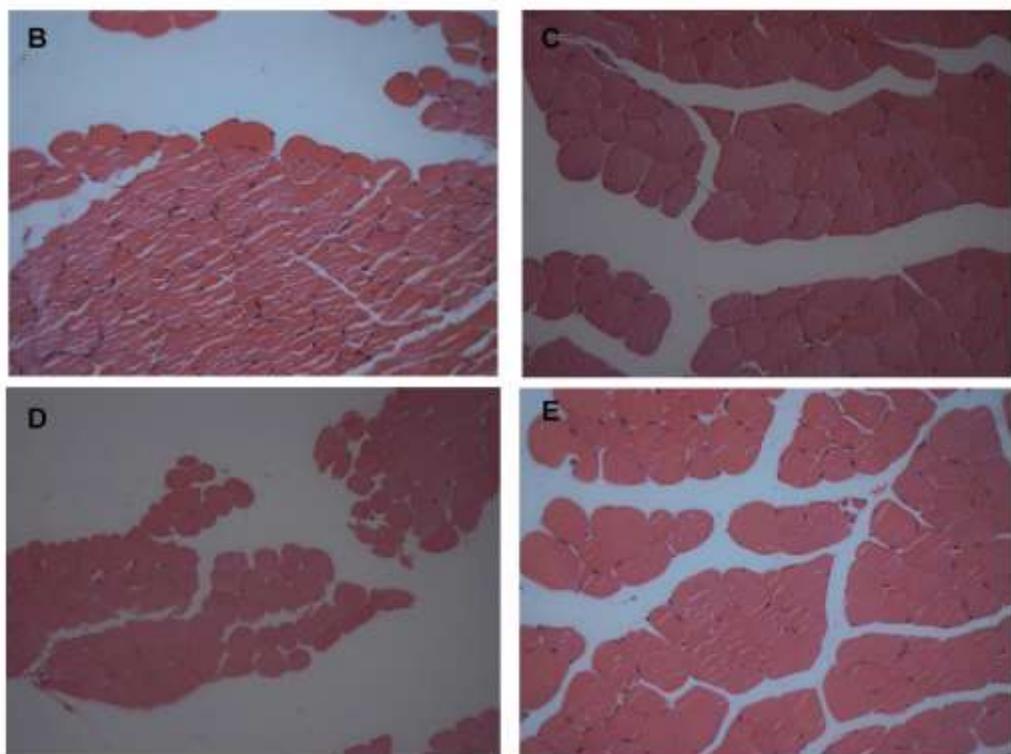
qPCR analyses: At initial disease stage, expression of GDF-8 and GDF-15 was not altered between groups, and GDF-11 levels were increased in CIA group compared to CO animals (p=0.004; Figure 6A, 6B and 6C). At established disease stage, GDF-8 expression was lower (p=0.010) and GDF-11 expression tended to be higher in CIA group, compared to CO group (p=0.07). GDF-15 expression was not altered between groups (Figure 6D, 6E and 6F).

Figure 6 **(A)** GDF-8, **(B)** GDF-11 and **(C)** GDF-15 RNA expression showed at initial arthritis **(A, B, C)** and established arthritis **(D, E, F);** GDF-8 and GDF-15 did not presented differences between groups at inicial arthritis; **(E)** GDF-11 was significantly higher compared to CO and HC groups (p<0.05) at initial arthritis. **(D)** GDF-8 RNA expression at established arthritis was significant lower at CIA compared to CO; **(E)** GDF-11 and **(F)** GDF-15 did not presented differences between groups (p>0.05). Data are expressed as median (relative expression to GAPDH) $*$ 100 ± SEM.

HC: Healthy animal; CO: Animals without induction; CIA: Animals induced.
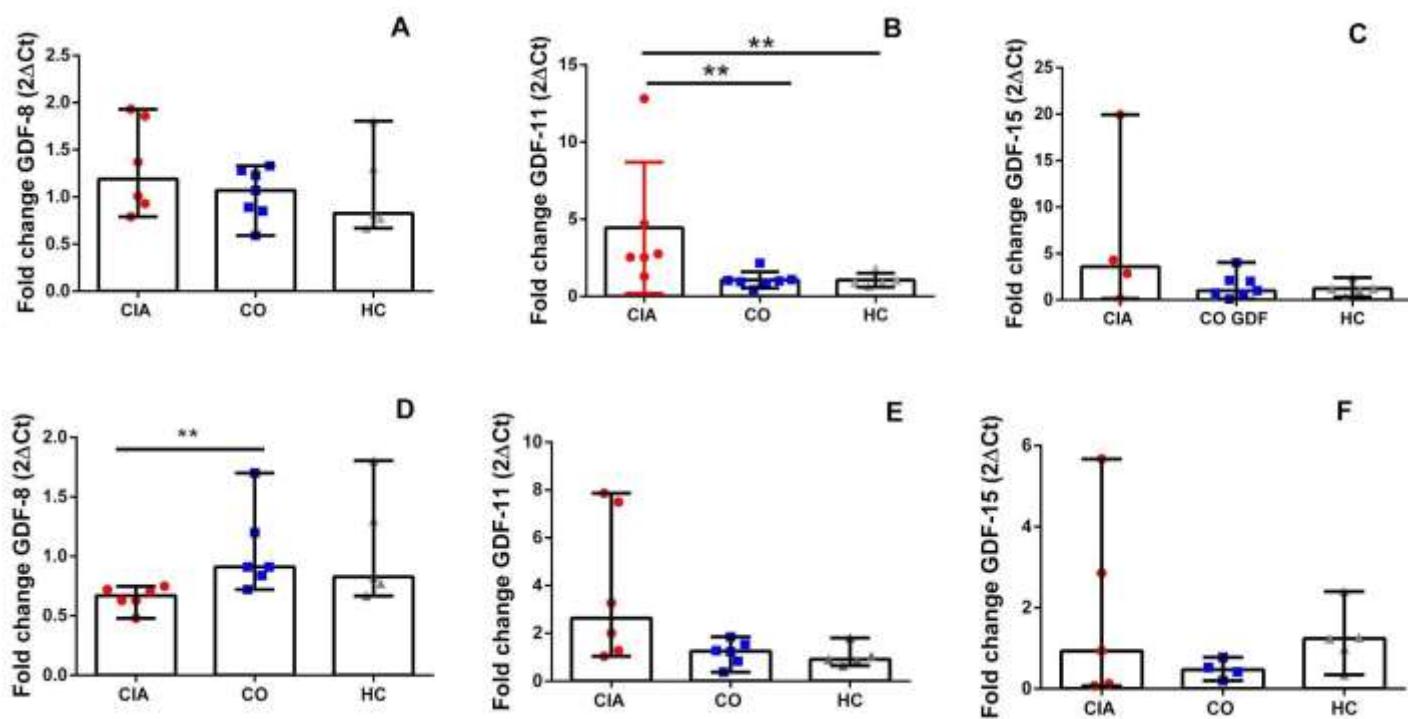
Association analyses: Higher GDF11 expression was negatively associated with grip strength at early disease (r=-77; p=0.072) (Figure 7)

Figure 7 Association between mRNA expression levels of GDF-11 and grip strength at early disease in CIA group (r=-0,77; p=0.072). Correlation was made using Spearman's correlation test.
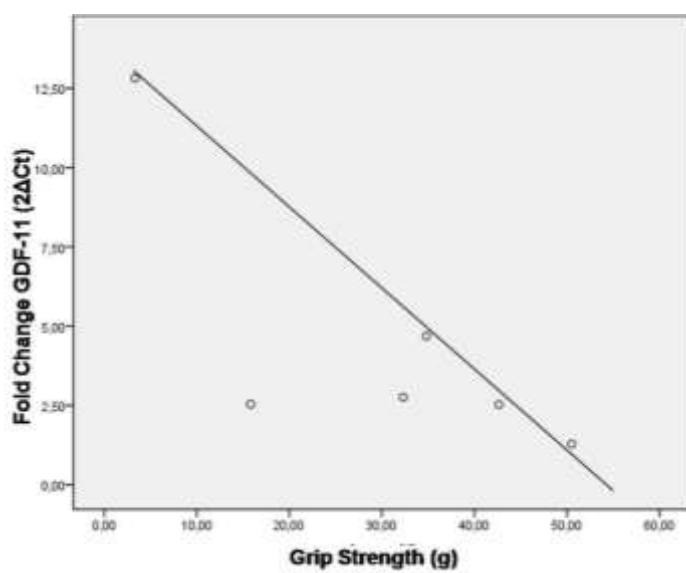
Discussion

RA patients present deficits in muscle area, density and strength, compared to healthy individuals, which impairs daily work and impacts in a socioeconomic manner [2,40]. Due to this issue, we investigated if grow differentiate factors 8, 11 and 15 could be involved in loss of muscle and strength in murine model CIA. Our findings suggests that CIA animals presented muscle atrophy accompanied by loss of grip strength and as well as alterations in muscle GDF mRNA expressions along the development of the disease. Thus, TGF-b family represents a fine research source to investigate changes in muscle mass in chronic diseases since TGF-β ligands may play a relevant role in homeostasis between protein degradation and protein synthesis and contribute to muscle physiology [17].

The temporal development muscle atrophy in collagen-induced arthritis was described by Filippin et al and, based on this study, we proposed two time points to evaluate the expression of GDFs in CIA and CO mice[38.] The days 25 and 50 after the disease induction would permit the evaluation of muscle impairment in initial and established disease, respectively. Disease score and hind paw edema were higher at CIA animals in both time points (25 and 50 days) as previously described in the literature and it is possible to see that the mild disease present in day 25 gets progressively more severe until day 50. Also histopathological score confirmed that animals induced in day 0 develop arthritis, as expected[38].

Muscle strength, at initial disease, was reduced in CIA group, compared to CO group and this loss of strength was aggravated in established CIA. Alabarse et al also described grip strength loss 25 days after the disease induction in CIA model[41]. The alterations in muscle strength are similar to those observed in humans, since it was demonstrated that RA patients have lower muscle strength, compared to controls, and that greater joint destruction is associated with greater muscle deficits[13]. In an ongoing study by our group, Ra patients have a statistical decrease in quadriceps muscle strength if compared with healthy controls (data not published).

The evaluation of muscle CSA demonstrated that CIA mice have decreased muscle area when the disease is established, but not in initial disease. Additionally, muscle weight and sarcoplasmic ratio presented reduction in established disease. Our results corroborate with previous literature that reported muscle CSA reduction after 45 days of the disease induction, when muscle atrophy was evaluated in various time points of CIA development[38]

In RA patients, decreased muscle mass was observed, which together with lower muscle quality and reduced mechanical loading to bone, lead to deficits in bone structure and contributes to reduce physical activity and increased risk of falls and fractures in these patients[42,43]. Although we did not find reduced myofiber CSA in CIA mice with initial arthritis, in the 25th day of the disease these animals had significant loss on muscle strength, which may mean a compromised muscle functionality without loss of area. Thus, a reduce in muscle weight were seeded at established disease and also in sarcoplasmic ratio, which support the loss in CSA decreased at CIA group at 50 days after disease onset.

Muscle loss caused by chronic disease has been demonstrated in several studies and during inflammation state[44–46]; A few studies already been reported molecular mechanisms for muscle impairment, which are probably related to by pro-inflammatory cytokines, such as TNF-α and IL-6, however this mechanisms differ when the muscle wasting is consequence of the disuse induced by pain, the inflammatory state of disease, or both[49,50]. Thus, we investigated if GDF-8, 11 and 15 participate at muscle alterations in initial arthritis, before the presence of muscle atrophy, and in established arthritis, when the muscle impairment becomes severe.

Our findings suggested that, in initial arthritis, GDF-11 concentrations raises in CIA group and tended to remain increased at established arthritis compared to CO group. Similarly, Egerman et al. reported increased GDF-11 serum levels and increase GDF-11 mRNA in skeletal muscle of rats, which undergo sarcopenia when they age [27,51]. Also, GDF-11 was described as a suppressor of skeletal muscle regeneration and its supraphysiologic administration could led to a cachexia state[27,34]. All of these findings agrees with our results which shows a role of GDF-11 as a skeletal muscle negative modulator.

The GDF-8 has no expression alteration at initial disease, but its muscle levels are decreased as arthritis progresses, comparing CIA and CO mice. This result agrees with a publish study that reported low levels of GDF-8 mRNA expression in serum and muscle of animals with adjuvant-induce arthritis AIA[55] . Additionally, in RA patients in remission serum levels of GDF-8 are diminished[52]. Regarding GDF-15 previous studies reported its association with loss of body weight, since GDF-15 modulates appetite and has a pivotal role in anorexia[34]. However, our results show no difference in GDF-15 mRNA expression between CIA and CO groups in both time points (25 and 50 days). It has been reported that CIA model do not lead to anorexia, as 65 days after the disease induction, CIA animals show intake food rate very similar to control animals[41].

In our study, muscle GDF-11 expression increases early in the development of arthritis, when strength is already affected. When muscle CSA diminishes, and therefore atrophy is established, GDF-11 has a tendency to increase as well. These results demonstrate a link among GDF-11 activity and important muscle features, which are related to clinical outcomes and the patient survival skills. Additionally, we found a negative correlation between GDF-11 mRNA expression and muscle strength, which support a connection between increased GDF-11 muscle levels and the loss of strength in the very beginning of arthritis disease. Otherwise, there are no changes in GDF-8 expression in initial disease, but only in established disease. It is likely that GDF-8 expression decreases when muscle loss established as a compensatory mechanism to avoid severe muscle impairment[50.53]. Some studies, in animal models and in RA patients, suggest that there is a possible compensatory mechanism for the muscle wasting of RA chronic inflammation [54].

As we know, this is the first study that analyzed the role of GDF 8, 11 and 15 in muscle features along the development of CIA and associate GDF-11 levels with decrease of grip strength. Taking our findings together, we can suggest that TGF-β family, especially GDF-8 and 11, may have a critical role at muscle outcomes in an AR murine model, and that they can be involved in muscle atrophy and loss of strength.

Perspectives

An expression analyses of GDF-11, 8 and 15 in human muscle tissue is still needed and a profound study of these molecules and its association with grip strength and muscle loss. Also, in vitro analysis of TGF-beta family in myotube differentiation and growth can provide more evidence of its effect in muscle cells.

Author contribution

BJB and JMSS have contributed to planning, CIA model follow up, statistics analysis, scientific discussion, and writing. BJB, JMSS, and MF have contributed to planning, CIA follow up, statistics, scientific discussion, and writing. RP, TH, TK has contributed to CIA model follow up and experiments. RMX was the supervisor for all experiments and has contributed to planning, scientific discussion, and paper corrections.

Conflicts of interest

The authors have no conflict of interest to declare.

REFERENCES

1.   Turesson C, O'Fallon WM, Crowson CS, Gabriel SE, Matteson EL. Occurrence of extraarticular disease manifestations is associated with excess mortality in a community based cohort of patients with rheumatoid arthritis. *J Rheumatol* 2002;**29**:62–67.

2.   Young A, Koduri G. Extra-articular manifestations and complications of rheumatoid arthritis. *Best Pract Res Clin Rheumatol* 2007;**21**:907–27.

3.   Bouchard DR, Janssen I. Dynapenic-obesity and physical function in older adults. *Journals Gerontol - Ser A Biol Sci Med Sci* 2010;**65**:71–77.

4.   Manini TM, Clark BC. Dynapenia and aging: An update. *Journals Gerontol - Ser A Biol Sci Med Sci* 2012;**67 A**:28–40.

5.   Cawthon PM, Fox KM, Gandra SR, Delmonico MJ, Chiou CF, Anthony MS *et al.* Clustering of strength, physical function, muscle, and adiposity characteristics and risk of disability in older adults. *J Am Geriatr Soc* 2011;**59**:781–787.

6.   Cimmino MA, Salvarani C, Macchioni P, Montecucco C, Fossaluzza V, Mascia MT *et al.* Extra-articular manifestations in 587 Italian patients with rheumatoid arthritis. *Rheumatol Int* 2000;**19**:213–217.

7.   Andrews JS, Trupin L, Yelin EH, Hough CL, Covinsky KE, Katz PP. Frailty and reduced physical function go hand in hand in adults with rheumatoid arthritis: a US observational cohort study. *Clin Rheumatol* 2017;**36**:1031–1039.

8.   van Bokhorst-de van der Schueren MAE, Konijn NPC, Bultink IEM, Lems WF, Earthman CP, van Tuyl LHD. Relevance of the new pre-cachexia and cachexia definitions for patients with rheumatoid arthritis. *Clin Nutr* 2012;**31**:1008–10.

9.   Elkan A-C, Håkansson N, Frostegård J, Cederholm T, Hafström I. Rheumatoid cachexia is associated with dyslipidemia and low levels of atheroprotective natural antibodies against phosphorylcholine but not with dietary fat in patients with rheumatoid arthritis: a cross-sectional study. *Arthritis Res Ther* 2009;**11**:R37.

10.   Munro R, Capell H. Prevalence of low body mass in rheumatoid arthritis:

association with the acute phase response. *Ann Rheum Dis* 1997;**56**:326–329.

11.   Roubenoff R, Roubenoff RA, Ward LM, Holland SM, Hellmann DB. Rheumatoid cachexia: depletion of lean body mass in rheumatoid arthritis. Possible association with tumor necrosis factor. *J Rheumatol* 1992;**19**:1505–10.

12.   Elkan A-C, Engvall I-L, Cederholm T, Hafström I. Rheumatoid cachexia, central obesity and malnutrition in patients with low-active rheumatoid arthritis: feasibility of anthropometry, Mini Nutritional Assessment and body composition techniques. *Eur J Nutr* 2009;**48**:315–22.

13.   Baker JF, Von Feldt J, Mostoufi-Moab S, Noaiseh G, Taratuta E, Kim W *et al.* Deficits in muscle mass, muscle density, and modified associations with fat in rheumatoid arthritis. *Arthritis Care Res (Hoboken)* 2014;**66**:1612–8.

14.   Sakuma K, Yamaguchi A. Sarcopenia and cachexia: the adaptations of negative regulators of skeletal muscle mass. *J Cachexia Sarcopenia Muscle* 2012;**3**:77–94.

15.   Glass DJ. Skeletal muscle hypertrophy and atrophy signaling pathways. *Int J Biochem Cell Biol* 2005;**37**:1974–1984.

16.   Ferraccioli G, Bracci-Laudiero L, Alivernini S, Gremese E, Tolusso B, De Benedetti F. Interleukin-1β and interleukin-6 in arthritis animal models: roles in the early phase of transition from acute to chronic inflammation and relevance for human rheumatoid arthritis. *Mol Med* 2010;**16**:552–7.

17.   Sartori R, Gregorevic P, Sandri M. TGFβ and BMP signaling in skeletal muscle: potential significance for muscle-related disease. *Trends Endocrinol Metab* 2014;**25**:464–471.

18.   Johnen H, Lin S, Kuffner T, Brown DA, Wang V, Tsai W *et al.* Tumor-induced anorexia and weight loss are mediated by the TGF-b superfamily cytokine MIC-1. *Nat Med Vol* 2007;**13**.

19.   Roubenoff R, Freeman LM, Smith DE, Abad LW, Dinarello CA, Kehayias JJ. Adjuvant arthritis as a model of inflammatory cachexia. *Arthritis Rheum* 1997;**40**:534–539.

20.   Dayer J-M. Interleukin 1 or tumor necrosis factor-alpha: which is the real target in rheumatoid arthritis? *J Rheumatol Suppl* 2002;**65**:10–5.

21.   Massagué J. TGFβ signalling in context. *Nat Rev Mol Cell Biol* 2012;**13**:616–30.

22.   Beggs ML, Nagarajan R, Taylor-Jones JM, Nolen G, MacNicol M, Peterson

CA. Alterations in the TGFβ signaling pathway in myogenic progenitors with age. *Aging Cell* 2004;**3**:353–361.

23.   Carlson ME, Conboy MJ, Hsu M, Barchas L, Jeong J, Agrawal A *et al.* Relative roles of TGF-β1 and Wnt in the systemic regulation and aging of satellite cell responses. *Aging Cell* 2009;**8**:676–689.

24.   McPherron AC, Lawler AM, Lee SJ. Regulation of anterior/posterior patterning of the axial skeleton by growth/differentiation factor 11. *Nat Genet* 1999;**22**:260–4.

25.   Trendelenburg AU, Meyer A, Rohner D, Boyle J, Hatakeyama S, Glass DJ. Myostatin reduces Akt/TORC1/p70S6K signaling, inhibiting myoblast differentiation and myotube size. *Am J Physiol Physiol* 2009;**296**:C1258–C1270.

26.   Lee SJ, Lee YS, Zimmers TA, Soleimani A, Matzuk MM, Tsuchida K *et al.* Regulation of muscle mass by follistatin and activins. *Mol Endocrinol* 2010;**24**:1998–2008.

27.   Egerman MA, Cadena SM, Gilbert JA, Meyer A, Nelson HN, Swalley SE *et al.* GDF11 Increases with Age and Inhibits Skeletal Muscle Regeneration. *Cell Metab* 2015;**22**:164–74.

28.   Nyhäll-Wåhlin BM, Petersson IF, Nilsson JÅ, Jacobsson LTH, Turesson C, Ahlmén M *et al.* High disease activity disability burden and smoking predict severe extra-articular manifestations in early rheumatoid arthritis. *Rheumatology* 2009;**48**:416–420.

29.   Hill JJ, Davies M V, Pearson AA, Wang JH, Hewick RM, Wolfman NM *et al.* The myostatin propeptide and FLRG are inhibitory binding proteins of myostatin in normal serum. J.Biol.Chem. .

30.   McPherron AC, Lawler AM, Lee SJ. Regulation of skeletal muscle mass in mice by a new TGF-beta superfamily member. *Nature* 1997;**387**:83–90.

31.   Rodriguez J, Vernus B, Chelh I, Cassar-Malek I, Gabillard JC, Hadj Sassi A *et al.* Myostatin and the skeletal muscle atrophy and hypertrophy signaling pathways. *Cell Mol Life Sci* 2014;**71**:4361–71.

32.   Sinha M, Jang YC, Oh J, Khong D, Wu EY, Manohar R *et al.* Restoring systemic GDF11 levels reverses age-related dysfunction in mouse skeletal muscle. *Science* 2014;**344**:649–52.

33.   Loffredo FS, Steinhauser ML, Jay SM, Gannon J, Pancoast JR, Yalamanchi P *et al.* Growth differentiation factor 11 is a circulating factor that reverses age-related

cardiac hypertrophy. *Cell* 2013;**153**:828–839.

34.   Jones JE, Cadena SM, Gong C, Wang X, Chen Z, Wang SX *et al.* Supraphysiologic Administration of GDF11 Induces Cachexia in Part by Upregulating GDF15. *Cell Rep* 2018;**22**:3375.

35.   Emmerson PJ, Wang F, Du Y, Liu Q, Pickard RT, Gonciarz MD *et al.* The metabolic effects of GDF15 are mediated by the orphan receptor GFRAL. *Nat Med* 2017;**23**:1215–1219.

36.   Mullican SE, Lin-Schmidt X, Chin C-N, Chavez JA, Furman JL, Armstrong AA *et al.* GFRAL is the receptor for GDF15 and the ligand promotes weight loss in mice and nonhuman primates. *Nat Med* 2017;**23**:1150–1157.

37.   Brand DD, Latham KA, Rosloniec EF. Collagen-induced arthritis. *Nat Protoc* 2007;**2**:1269–1275.

38.   Filippin LI, Teixeira VN, Viacava PR, Lora PS, Xavier LL, Xavier RM. Temporal development of muscle atrophy in murine model of arthritis is related to disease severity. *J Cachexia Sarcopenia Muscle* 2013;**4**:231–8.

39.   Deacon RMJ. Measuring the strength of mice. *J Vis Exp* 2013 doi:10.3791/2610.

40.   Khurana R, Berney SM. Clinical aspects of rheumatoid arthritis. *Pathophysiology* 2005;**12**:153–165.

41.   Alabarse PVG, Lora PS, Silva JMS, Santo RCE, Freitas EC, de Oliveira MS *et al.* Collagen-induced arthritis as an animal model of rheumatoid cachexia. *J Cachexia Sarcopenia Muscle* 2018;**9**:603–612.

42.   Okano T, Inui K, Tada M, Sugioka Y, Mamoto K, Wakitani S *et al.* Loss of lean body mass affects low bone mineral density in patients with rheumatoid arthritis – results from the TOMORROW study. *Mod Rheumatol* 2017;1–7.

43.   Giles JT, Bartlett SJ, Andersen RE, Fontaine KR, Bathon JM. Association of body composition with disability in rheumatoid arthritis: impact of appendicular fat and lean tissue mass. *Arthritis Rheum* 2008;**59**:1407–15.

44.   Okiura T, Nagatomo F, Gu N, Taguchi Y, Morimatsu F, Ishihara A. Bone density of the femur and fiber cross-sectional area and oxidative enzyme activity of the tibialis anterior muscle in type II collagen-induced arthritic mice. *J Physiol Sci* 2008;**58**:221–227.

45.   Hartog A, Hulsman J, Garssen J. Locomotion and muscle mass measures in a murine model of collagen-induced arthritis. *BMC Musculoskelet Disord* 2009;**10**:59.

46.   Roubenoff R. Inflammatory and hormonal mediators of cachexia. *J Nutr* 1997;**127**:1014S–1016S.

47.   Castillero E, Nieto-Bona MP, Fernández-Galaz C, Martín AI, López-Menduiña M, Granado M *et al.* Fenofibrate, a PPARα agonist, decreases atrogenes and myostatin expression and improves arthritis-induced skeletal muscle atrophy. *Am J Physiol - Endocrinol Metab* 2011;**300**.

48.   Granado M, Priego T, Martín AI, Villanúa MÁ, López-Calderón A. Anti-inflammatory effect of the ghrelin agonist growth hormone-releasing peptide-2 (GHRP-2) in arthritic rats. *Am J Physiol - Endocrinol Metab* 2005;**288**.

49.   Granado M, Martín AI, Priego T, López-Calderón A, Villanúa MA. Tumour necrosis factor blockade did not prevent the increase of muscular muscle RING finger-1 and muscle atrophy F-box in arthritis rats. *J Endocrinol* 2006;**191**:319–326.

50.   de Oliveira Nunes Teixeira V, Filippin LI, Viacava PR, de Oliveira PG, Xavier RM. Muscle wasting in collagen-induced arthritis and disuse atrophy. *Exp Biol Med* 2013;**238**:1421–1430.

51.   Ibebunjo C, Chick JM, Kendall T, Eash JK, Li C, Zhang Y *et al.* Genomic and proteomic profiling reveals reduced mitochondrial function and disruption of the neuromuscular junction driving rat sarcopenia. *Mol Cell Biol* 2013;**33**:194–212.

52.   Kerschan-Schindl K, Ebenbichler G, Föeger-Samwald U, Leiss H, Gesslbauer C, Herceg M *et al.* Rheumatoid arthritis in remission : Decreased myostatin and increased serum levels of periostin. *Wien Klin Wochenschr* 2019;**131**:1–7.

53.   Amthor H, Otto A, Vulin A, Rochat A, Dumonceaux J, Garcia L *et al.* Muscle hypertrophy driven by myostatin blockade does not require stem/precursor-cell activity. *Proc Natl Acad Sci U S A* 2009;**106**:7479–84.

54.   Little RD, Prieto-Potin I, Pérez-Baos S, Villalvilla A, Gratal P, Cicuttini F *et al.* Compensatory anabolic signaling in the sarcopenia of experimental chronic arthritis. *Sci Rep* 2017;**7**:1–11.

55.   Li W, Wang W, Liu L, Qu R, Chen X, Qiu C, Li J, Hayball J, Liu L, Chen J, Wang X, Pan X, Zhao Y *et al*. GDF11 antagonizes TNF-α–induced inflammation and protects against the development of inflammatory arthritis in mice. FASEB J. 2019 Mar;33(3):3317-3329. FASEB J. 2019 Mar;33(3):3317-3329.

3 CONCLUSÕES E PERSPECTIVAS

Os resultados obtidos no estudo mostram o papel dos fatores de crescimento e diferenciação 8, 11 e 15 na perda muscular e perda de força na artrite reumatoide. Segundo os dados obtidos, sugere-se um efeito negativo do GDF-11 na perda muscular e sua associação com a perda de força. O GDF-8 aparenta ter uma expressão diminuída ao longo do desenvolvimento da AR e, por sua vez, o GDF-15 não parece ter envolvimento com a doença. Com base nisso, e nas dificuldades apresentadas em tratar a perda muscular que os pacientes apresentam na prática clínica, a família TGF-β pode ser um alvo em potencial para estudo e, mais adiante, como um biomarcador para avaliação de déficits musculares.

REFERENCIAS

ABBOTT, Joel D e MORELAND, Larry W. **Rheumatoid arthritis: developing pharmacological therapies**. Expert Opinion on Investigational Drugs, v. 13, n. 8, p. 1007–1018, 24 Ago 2004. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/15268638>.

ALETAHA, Daniel e ALASTI, Farideh e SMOLEN, Josef S. **Rheumatoid factor, not antibodies against citrullinated proteins, is associated with baseline disease activity in rheumatoid arthritis clinical trials**. Arthritis Research and Therapy, v. 17, n. 1, 26 Ago 2015.

AMTHOR, Helge e colab. **Muscle hypertrophy driven by myostatin blockade does not require stem/precursor-cell activity.** Proceedings of the National Academy of Sciences of the United States of America, v. 106, n. 18, p. 7479–84, 5 Maio 2009. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/19383783>. Acesso em: 5 jul 2018.

ANQUETIL, Florence e colab. **IgM and IgA Rheumatoid Factors Purified from Rheumatoid Arthritis Sera Boost the Fc Receptor– and Complement-Dependent Effector Functions of the Disease-Specific Anti–Citrullinated Protein Autoantibodies**. The Journal of Immunology, v. 194, n. 8, p. 3664–3674, 15 Abr 2015.

BAKER, Joshua F e colab. **Deficits in muscle mass, muscle density, and modified associations with fat in rheumatoid arthritis.** Arthritis care & research, v. 66, n. 11, p. 1612–8, Nov 2014. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/24664868>. Acesso em: 5 jul 2018.

BARRA, Lillian e colab. **Lack of seroconversion of rheumatoid factor and anti-cyclic citrullinated peptide in patients with early inflammatory arthritis: a systematic literature review.** Rheumatology (Oxford, England), v. 50. n. 2, p. 311–6, Fev 2011. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/20621983>. Acesso em: 17 nov 2019.

BURNHAM, R e RUSSELL, A S. **Nutritional status in patients with rheumatoid arthritis.** Annals of the rheumatic diseases, v. 45, n. 9, p. 788–9, Set 1986. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/3767470>. Acesso em: 4 fev 2019.

CHOY, Ernest. **Understanding the dynamics: Pathways involved in the pathogenesis of rheumatoid arthritis**. Rheumatology (United Kingdom), v. 51, n. SUPPL.5, Jul 2012.

CUTOLO, Maurizio e KITAS, George D. e VAN RIEL, Piet L.C.M. **Burden of disease in treated rheumatoid arthritis patients: Going beyond the joint**. Seminars in Arthritis and Rheumatism, v. 43, n. 4, p. 479–488, Fev 2014. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/24080116>. Acesso em: 4 fev 2019.

DE OLIVEIRA NUNES TEIXEIRA, Vivian e colab. **Muscle wasting in collagen-induced arthritis and disuse atrophy**. Experimental Biology and Medicine, v. 238, n. 12, p. 1421–1430. 1 Dez 2013. Disponível em: <http://journals.sagepub.com/doi/10.1177/1535370213505961>. Acesso em: 5 jul 2018.

EBRINGER, A e WILSON, C. **HLA molecules, bacteria and autoimmunity.** Journal of medical microbiology, v. 49, n. 4, p. 305–11, Abr 2000. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/10755623>. Acesso em: 17 nov 2019.

EGERMAN, Marc A. e colab. **GDF-11 Increases with Age and Inhibits Skeletal Muscle**

Regeneration. Cell Metabolism, v. 22, n. 1, p. 164–174, Jul 2015. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S1550413115002223>. Acesso em: 5 jul 2018.

EMMERSON, Paul J e colab. **The metabolic effects of GDF15 are mediated by the orphan receptor GFRAL**. Nature Medicine, v. 23, n. 10. p. 1215–1219, 28 Ago 2017. Disponível em: <http://www.nature.com/doifinder/10.1038/nm.4393>. Acesso em: 5 jul 2018.

FIRESTEIN, Gary S e MCINNES, Iain B. **Immunopathogenesis of Rheumatoid Arthritis.** Immunity, v. 46, n. 2, p. 183–196, 21 Fev 2017. Disponível em: <http://dx.doi.org/10.1016/j.immuni.2017.02.006>. Acesso em: 15 ago 2017.

FLEMING, A. e CORBETT, Mary e CROWN, June M. **Prognostic value of early features in rheumatoid disease**. British Medical Journal, v. 1, n. 6020. p. 1243–1245, 22 Maio 1976.

FUKUDA, Wataru e colab. **Contribution of rheumatoid arthritis disease activity and disability to rheumatoid cachexia.** Modern rheumatology, v. 20. n. 5, p. 439–43, Out 2010. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/20508962>. Acesso em: 17 nov 2019.

GONZALEZ, Angel e colab. **Mortality trends in rheumatoid arthritis: The role of rheumatoid factor**. Journal of Rheumatology, v. 35, n. 6, p. 1009–1014, Jun 2008.

GREGERSEN, Peter K. e SILVER, Jack e WINCHESTER, Robert J. **The shared epitope hypothesis. an approach to understanding the molecular genetics of susceptibility to rheumatoid arthritis**. Arthritis & Rheumatism, v. 30. n. 11, p. 1205–1213, 1987.

GROBET, Luc e colab. **Modulating skeletal muscle mass by postnatal, muscle-specific inactivation of the myostatin gene**. genesis, v. 35, n. 4, p. 227–238, Abr 2003. Disponível em: <http://doi.wiley.com/10.1002/gene.10188>. Acesso em: 5 jul 2018.

HONDA, Kenya e LITTMAN, Dan R. **The microbiome in infectious disease and inflammation.** Annual review of immunology, v. 30. p. 759–95, 2012. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/22224764>. Acesso em: 17 nov 2019.

HU, Sung-Lin e colab. **Myostatin Promotes Interleukin-1β Expression in Rheumatoid Arthritis Synovial Fibroblasts through Inhibition of miR-21-5p.** Frontiers in immunology, v. 8, p. 1747, 2017. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/29276516>. Acesso em: 8 out 2019.

HUFFMAN, Kim M e colab. **Molecular alterations in skeletal muscle in rheumatoid arthritis are related to disease activity, physical inactivity, and disability.** Arthritis research & therapy, v. 19, n. 1, p. 12, 23 Jan 2017. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/28114971>. Acesso em: 17 nov 2019.

JACKMAN, Robert W e KANDARIAN, Susan C. **The molecular basis of skeletal muscle atrophy.** American journal of physiology. Cell physiology, v. 287, n. 4, p. C834-43, 2004. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/15355854>.

JOHNEN, Heiko e colab. **Tumor-induced anorexia and weight loss are mediated by the TGF-b superfamily cytokine MIC-1**. NATURE MEDICINE VOLUME, v. 13, 2007. Disponível em: <http://www.nature.com/naturemedicine>. Acesso em: 5 jul 2018.

JONES, Juli E e colab. **Supraphysiologic Administration of GDF-11 Induces Cachexia in Part by Upregulating GDF15.** Cell reports, v. 22, n. 12, p. 3375, 20 Mar 2018. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/29562191>. Acesso em: 5 jul 2018.

KERSCHAN-SCHINDL, Katharina e colab. **Rheumatoid arthritis in remission : Decreased**

myostatin and increased serum levels of periostin. Wiener klinische Wochenschrift, v. 131, n. 1–2, p. 1–7, Jan 2019. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/30171335>. Acesso em: 8 out 2019.

KHURANA, Ritu e BERNEY, Seth Mark. **Clinical aspects of rheumatoid arthritis**. Pathophysiology, v. 12, n. 3, p. 153–165, 2005.

LEMMEY, Andrew B. e colab. **Tight control of disease activity fails to improve body composition or physical function in rheumatoid arthritis patients**. Rheumatology, v. 55, n. 10. p. 1736–1745, 1 Out 2016. Disponível em: <https://academic.oup.com/rheumatology/article-lookup/doi/10.1093/rheumatology/kew243>. Acesso em: 5 jul 2018.

LENZ, Tobias L. e colab. **Widespread non-additive and interaction effects within HLA loci modulate the risk of autoimmune diseases**. Nature Genetics, v. 47, n. 9, p. 1085–1090. 27 Ago 2015.

LI, Weiwei e colab. **GDF-11 antagonizes TNF-α–induced inflammation and protects against the development of inflammatory arthritis in mice**. The FASEB Journal, v. 33, n. 3, p. 3317–3329, Mar 2019.

LITTLE, Robert D. e colab. **Compensatory anabolic signaling in the sarcopenia of experimental chronic arthritis**. Scientific Reports, v. 7, n. 1, p. 1–11, 2017.

LU, Qiong e colab. **GDF-11 Inhibits Bone Formation by Activating Smad2/3 in Bone Marrow Mesenchymal Stem Cells**. Calcified Tissue International, v. 99, n. 5, p. 500–509, 9 Nov 2016. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/27395058>. Acesso em: 5 jul 2018.

LUNDBERG, Ingrid E e NADER, Gustavo A. **Molecular effects of exercise in patients with inflammatory rheumatic disease.** Nature clinical practice. Rheumatology, v. 4, n. 11, p. 597–604, Nov 2008. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/18839010>. Acesso em: 8 out 2019.

M., Stevenson e colab. **Adalimumab, etanercept, infliximab, certolizumab pegol, golimumab, tocilizumab and abatacept for the treatment of rheumatoid arthritis not previously treated with disease-modifying antirheumatic drugs and after the failure of conventional disease-modifyin**. Health Technology Assessment, v. 20. n. 35, p. 1–610. 2016. Disponível em: <http://www.embase.com/search/results?subaction=viewrecord&from=export&id=L610318279>. Acesso em: 17 nov 2019.

MASSAGUÉ, Joan. **TGFβ signalling in context.** Nature reviews. Molecular cell biology, v. 13, n. 10. p. 616–30. Out 2012. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/22992590>. Acesso em: 5 jul 2018.

MASSAGUÉ, Joan e SEOANE, Joan e WOTTON, David. **Smad transcription factors.** Genes & development, v. 19, n. 23, p. 2783–810. 1 Dez 2005. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/16322555>. Acesso em: 5 jul 2018.

MOTA, Licia Maria Henrique Da e colab. **Posicionamento sobre o uso de tofacitinibe no algoritmo do Consenso 2012 da Sociedade Brasileira de Reumatologia para o tratamento da artrite reumatoide**. Revista Brasileira de Reumatologia, v. 55, n. 6, p. 512–521, 1 Nov 2015.

MULLICAN, Shannon E e colab. **GFRAL is the receptor for GDF15 and the ligand promotes weight loss in mice and nonhuman primates.** Nature medicine, v. 23, n. 10. p. 1150–1157, Out 2017. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/28846097>. Acesso em: 17 nov 2019.

MUNRO, R. e CAPELL, H. **Prevalence of low body mass in rheumatoid arthritis: association with the acute phase response**. Annals of the Rheumatic Diseases, v. 56, n. 5, p. 326–329, 1 Maio 1997. Disponível em: <http://ard.bmj.com/cgi/doi/10.1136/ard.56.5.326>.

NAKASHIMA, M e colab. **Expression of growth/differentiation factor 11, a new member of the BMP/TGFbeta superfamily during mouse embryogenesis.** Mechanisms of development, v. 80. n. 2, p. 185–9, Fev 1999. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/10072786>. Acesso em: 5 jul 2018.

NYHÄLL-WÅHLIN, Britt Marie e colab. **High disease activity disability burden and smoking predict severe extra-articular manifestations in early rheumatoid arthritis**. Rheumatology, v. 48, n. 4, p. 416–420. 2009.

POGGIOLI, Tommaso e colab. **Circulating Growth Differentiation Factor 11/8 Levels Decline With AgeNovelty and Significance**. Circulation Research, v. 118, n. 1, p. 29–37, 8 Jan 2016. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/26489925>. Acesso em: 5 jul 2018.

ROUBENOFF, Ronenn e colab. **Adjuvant arthritis as a model of inflammatory cachexia**. Arthritis and Rheumatism, v. 40. n. 3, p. 534–539, 1997.

ROUBENOFF, Ronenn e colab. **Rheumatoid cachexia: Cytokine-driven hypermetabolism accompanying reduced body cell mass in chronic inflammation**. Journal of Clinical Investigation, v. 93, n. 6, p. 2379–2386, 1994.

SABHARWAL, U K e colab. **Activation of the classical pathway of complement by rheumatoid factors. Assessment by radioimmunoassay for C4.** Arthritis and rheumatism, v. 25, n. 2, p. 161–7, Fev 1982. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/7066047>. Acesso em: 17 nov 2019.

SAKUMA, Kunihiro e YAMAGUCHI, Akihiko. **Sarcopenia and cachexia: the adaptations of negative regulators of skeletal muscle mass.** Journal of cachexia, sarcopenia and muscle, v. 3, n. 2, p. 77–94, Jun 2012. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/22476916>. Acesso em: 17 nov 2019.

SARTORI, Roberta e GREGOREVIC, Paul e SANDRI, Marco. **TGFβ and BMP signaling in skeletal muscle: potential significance for muscle-related disease**. Trends in Endocrinology & Metabolism, v. 25, n. 9, p. 464–471, 1 Set 2014a. Disponível em: <https://www.sciencedirect.com/science/article/pii/S1043276014001015?via%3Dihub>. Acesso em: 5 jul 2018.

SARTORI, Roberta e GREGOREVIC, Paul e SANDRI, Marco. **TGFβ and BMP signaling in skeletal muscle: Potential significance for muscle-related disease**. Trends in Endocrinology and Metabolism, v. 25, n. 9, p. 464–471, 2014b. Disponível em: <http://dx.doi.org/10.1016/j.tem.2014.06.002>.

SCHAFER, Marissa J. e colab. **Quantification of GDF-11 and Myostatin in Human Aging and Cardiovascular Disease**. Cell Metabolism, v. 23, n. 6, p. 1207–1215, 14 Jun 2016. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/27304512>. Acesso em: 5 jul 2018.

SCHER, Jose U. e colab. **Decreased bacterial diversity characterizes the altered gut microbiota in patients with psoriatic arthritis, resembling dysbiosis in inflammatory bowel disease**. Arthritis and Rheumatology, v. 67, n. 1, p. 128–139, 1 Jan 2015.

SCOTT, David L e WOLFE, Frederick e HUIZINGA, Tom WJ. **Rheumatoid arthritis**. The Lancet, v. 376, n. 9746, p. 1094–1108, Set 2010. Disponível em: <http://ac.els-

cdn.com/S0140673610608264/1-s2.0-S0140673610608264-main.pdf?_tid=13b3db3c-81f2-11e7-b493-00000aab0f6c&acdnat=1502826417_c19b5b79ef4502cfe215dc935913f40c>. Acesso em: 15 ago 2017.

SENNA, Erika Rodrigues e colab. **Prevalence of rheumatic diseases in Brazil: a study using the COPCORD approach.** The Journal of rheumatology, v. 31, n. 3, p. 594–7, Mar 2004. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/14994410>. Acesso em: 1 fev 2019.

SHARMA, M e colab. **Myostatin in muscle growth and repair.** Exercise and sport sciences reviews, v. 29, n. 4, p. 155–8, Out 2001. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/11688787>. Acesso em: 5 jul 2018.

SINGH, Jasvinder A e colab. **North Carolina; 16 Elizabeth Tindall, MD: Rheumatology Consultants of Oregon**. [S.d.]. Disponível em: <http://onlinelibrary.wiley.com/doi/>. Acesso em: 17 nov 2019.

SINHA, Manisha e colab. **Restoring systemic GDF-11 levels reverses age-related dysfunction in mouse skeletal muscle.** Science (New York, N.Y.), v. 344, n. 6184, p. 649–52, 9 Maio 2014. Disponível em: <http://www.sciencemag.org/cgi/doi/10.1126/science.1251152>. Acesso em: 5 jul 2018.

SMOLEN, Josef S. e colab. **EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2016 update**. Annals of the Rheumatic Diseases, v. 76, n. 6, p. 960–977, 2017.

SMOLEN, Josef S. e BREEDVELD, Ferdinand C. e colab. **Treating rheumatoid arthritis to target: 2014 update of the recommendations of an international task force**. Annals of the Rheumatic Diseases, v. 75, n. 1, p. 3–15, 1 Jan 2016.

SMOLEN, Josef S e ALETAHA, Daniel e MCINNES, Iain B. **Rheumatoid arthritis**. The Lancet, v. 388, n. 10055, p. 2023–2038, 22 Out 2016. Disponível em: <https://www.sciencedirect.com/science/article/pii/S0140673616301738?via%3Dihub>. Acesso em: 5 jul 2018.

SU, Chen-Ming e colab. **Myostatin induces tumor necrosis factor-α expression in rheumatoid arthritis synovial fibroblasts through the PI3K-Akt signaling pathway.** Journal of cellular physiology, v. 234, n. 6, p. 9793–9801, Jun 2019. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/30378113>. Acesso em: 17 nov 2019.

TOBÓN, Gabriel J e YOUINOU, Pierre e SARAUX, Alain. **The environment, geo-epidemiology, and autoimmune disease: Rheumatoid arthritis.** Journal of autoimmunity, v. 35, n. 1, p. 10–4, Ago 2010. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/20080387>. Acesso em: 8 out 2019.

TRACEY, K. J. e colab. **Metabolic effects of cachectin/tumor necrosis factor are modified by site of production. Cachectin/tumor necrosis factor-secreting tumor in skeletal muscle induces chronic cachexia, while implantation in brain induces predominately acute anorexia**. Journal of Clinical Investigation, v. 86, n. 6, p. 2014–2024, 1990.

TRENDELENBURG, Anne Ulrike e colab. **Myostatin reduces Akt/TORC1/p70S6K signaling, inhibiting myoblast differentiation and myotube size**. American Journal of Physiology-Cell Physiology, v. 296, n. 6, p. C1258–C1270. Jun 2009. Disponível em: <http://www.physiology.org/doi/10.1152/ajpcell.00105.2009>. Acesso em: 5 jul 2018.

TURESSON, C. e colab. **Occurrence of extraarticular disease manifestations is associated with excess mortality in a community based cohort of patients with rheumatoid arthritis**. Journal of

Rheumatology, v. 29, n. 1, p. 62–67, 2002.

VAN BOKHORST-DE VAN DER SCHUEREN, Marian A E e colab. **Relevance of the new pre-cachexia and cachexia definitions for patients with rheumatoid arthritis.** Clinical nutrition (Edinburgh, Scotland), v. 31, n. 6, p. 1008–10. Dez 2012. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/22695407>. Acesso em: 8 out 2019.

VAN GAALEN, Floris A e colab. **Association between HLA class II genes and autoantibodies to cyclic citrullinated peptides (CCPs) influences the severity of rheumatoid arthritis.** Arthritis and rheumatism, v. 50. n. 7, p. 2113–21, Jul 2004. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/15248208>. Acesso em: 17 nov 2019.

VIATTE, Sebastien e colab. **Association of HLA-DRB1 haplotypes with rheumatoid arthritis severity, mortality, and treatment response.** JAMA, v. 313, n. 16, p. 1645–56, 28 Abr 2015. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/25919528>. Acesso em: 17 nov 2019.

YOUNG, Adam e KODURI, Gouri. **Extra-articular manifestations and complications of rheumatoid arthritis.** Best practice & research. Clinical rheumatology, v. 21, n. 5, p. 907–27, Out 2007. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/17870035>. Acesso em: 8 out 2019.

ZHAO, Xiaoyan e colab. **Circulating immune complexes contain citrullinated fibrinogen in rheumatoid arthritis**. Arthritis Research and Therapy, v. 10. n. 4, 18 Ago 2008.

ANEXO A - GUIDELINES PARA SUBMISSÃO DE ARTIGO NA REVISTA JOURNAL OF CACHEXIA, SARCOPENIA AND MUSCLE

Journal of cachexia, sarcopenia and muscle

Monika Diek, Charité - Universitätsmedizin Berlin, Campus Virchow-Klinikum, Augustenburger Platz 1, 13353 Berlin, Germany, Email: jcsm.editorialoffice@wiley.com, Tel: +49 (0)30-450 553 407.

Author Guidelines

Aims and Scope

The Journal of Cachexia, Sarcopenia and Muscle is an open access, peer-reviewed international journal dedicated to publishing materials that are related to cachexia and sarcopenia, as well as to body composition and its physiological and pathophysiological changes during the lifespan and in response to different illnesses from all fields of the life sciences.

The term cachexia describes involuntary weight loss that is observed in the course of many chronic diseases, and is one of the most debilitating and life-threatening aspects of various illnesses at advanced stages. Cachexia, wasting syndromes and sarcopenia are becoming a concerning challenge for an increasing number of patients, their relatives and the medical teams caring for them. The Journal of Cachexia, Sarcopenia and Muscle aims to offer a reliable resource to all professionals who are interested in related research or who are involved in the clinical care of affected patients, for example those suffering from AIDS, cancer, chronic heart failure, chronic lung disease, liver cirrhosis, chronic kidney failure, rheumatoid arthritis, or sepsis.

Alterations in body composition, particularly those affecting skeletal muscle, are key elements in the ageing process and in the pathophysiology of several chronic illnesses. Sarcopenia, i.e. loss of functional muscle mass without weight loss, is part of the ageing process and may play a role in reduced physical performance, falls, and disability. Studies on the functional importance of fat tissue and mechanisms leading to lipolysis are equally of interest as are studies on mechanisms of muscle wasting.

The pathophysiology of cachexia involves a complex interaction between disease and body. Consequently, numerous potential therapeutic approaches are being considered and developed. Diagnostic and assessment approaches also involve researchers and clinicians seeking better screening and evaluation options and enhanced biomarkers through validated complementary investigations. This makes the Journal of Cachexia, Sarcopenia and Muscle a reliable resource of information for physicians, biochemists, biologists, dieticians, pharmacologists, and students dealing with cachexia, wasting and sarcopenia in various diseases.

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• Book chapter

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