Universidade Federal de São Carlos
CENTRO DE CIÊNCIAS BIOLÓGICAS E DA SAÚDE
PROGRAMA DE PÓS-GRADUAÇÃO EM FISIOTERAPIA

TESE DE DOUTORADO

RELAÇÃO DA RIGIDEZ E DO TOQUE ARTICULAR COM A DIABETES MELLITUS DO TIPO 2 E A NEUROPATIA PERIFÉRICA

Jean de Paula Ferreira

São Carlos
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Jean de Paula Ferreira

Tese apresentada ao Programa de Pós-Graduação em Fisioterapia da Universidade Federal de São Carlos, como requisito parcial para obtenção de título de Doutor em Fisioterapia.

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Coorientadora: Proa Dra. Paula Regina Mendes Serrão
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Folha de Aprovação

Defesa de Tese de Doutorado do candidato Jean de Paula Ferreira, realizada em 20/01/2020.

Comissão Julgadora:

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Prof. Dr. Ricky Watari (USP)

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O Relatório de Defesa assinado pelos membros da Comissão Julgadora encontra-se arquivado junto ao Programa de Pós-Graduação em Fisioterapia.

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Resumo

A literatura aponta uma forte relação entre as alterações musculoesqueléticas e a incidência de diabetes mellitus tipo 2 (DM2). Ainda não está claro se a DM2 afeta as propriedades passivas do sistema musculoesquelético, aumentando a rigidez e prejudicando a função muscular destes indivíduos. **Objetivo:** Analisar se os torques concêntrico e isométrico podem distinguir indivíduos com DM2 e neuropatia diabética periférica (NDP) de indivíduos sem DM com a mesma idade e características antropométricas e também, analisar o torque e a rigidez passiva em indivíduos com DM2, com e sem NDP, nos movimentos de flexão e extensão do joelho e do tornozelo, comparados com indivíduos sem DM. **Métodos:** Oitenta e oito participantes foram analisados, sendo 29 controles, 59 DM2 (23 com e 36 sem NDP). O controle glicêmico foi determinado pela HbA1C e a NDP foi analisado utilizando um sistema baseado no protocolo Michigan Neuropathy Screening Instrument (MNSI). O nível sérico das citocinas inflamatórias IL-1β, TNF-α e IL-6 foi analisado através do método de enzyme-linked immunosorbent assay (*ELISA*). Os picos de torque identificadas na análise de componente principal (ACP) foram utilizadas para a análise de cluster (k-means). Uma análise de regressão múltipla foi aplicada para investigar os fatores associados com HbA1c, citocinas inflamatórias e o escore do MNSI. Para a análise do torque passivo, três grupos de indivíduos do sexo masculino (n=49), similares em relação à idade, foram analisados, sendo 17 com DM2 sem NDP, 15 com DM2 e NDP e 17 controles sem DM2. O torque passivo de flexão e extensão do joelho e tornozelo foi avaliado com um dinamômetro isocinético à 5°/s, 30°/s e 60°/s, e posteriormente foi calculado a rigidez passiva utilizando o registro dos torques passivo. **Resultados:** O torque concêntrico de flexão e extensão do joelho e isométrico de extensão do tornozelo caracteriza 88.59% da condição clínica dos grupos, formando o Cluster 1 (n=29 controles) e o Cluster 2 (n=59 DM2). Maiores percentuais de HbA1c foi associado com o menor torque concêntrico de flexão e extensão do joelho e isométrico de extensão do tornozelo e maiores níveis de TNF-α e IL-6 e maior score do MNSI. Em relação à análise da rigidez, indivíduos com DM2 e NDP apresentaram maior torque passivo de extensão do joelho (p<0.01) e assim também um torque passivo e rigidez aumentada durante a dorsiflexão e flexão plantar (p<0.04) a 5°/s, quando comparado com controles e indivíduos DM2 sem NDP. **Conclusão:** Torques concêntrico e isométrico do tornozelo podem diferenciar indivíduos com e sem DM2, mas não aqueles com e sem NDP. O torque reduzido nos movimentos do joelho e tornozelo estão associados com os sintomas da NDP, o pobre controle glicêmico e a inflamação subclínica aumentada. Indivíduos com DM2 e NDP apresentam maior rigidez e torque passivo do joelho e tornozelo, comparado com indivíduos DM2 sem NDP e controles sem DM2.

Palavra-chave: diabetes; músculo esquelético; citocinas; membros inferiores; tornozelo; joelho, rigidez articular

Abstract

The literature indicates a strong relationship between musculoskeletal losses and the incidence of type 2 diabetes mellitus (DM2), and it is also unclear whether DM2 affects the passive properties of the musculoskeletal system, increasing the muscle stiffness and impairing the function of these individuals. **Objective:** To analyze whether concentric and isometric torques can distinguish between individuals with DM2 and peripheral diabetic neuropathy (DPN) from individuals without DM2 with the same age and anthropometric characteristics, and also to analyze the passive torque and passive stiffness in DM2 individuals, with and without NDP at the knee and ankle flexion and extension compared to individuals without DM. **Methods:** Of the 88 participants, 29 were controls, 59 with DM2 (23 with and 36 without DPN). Glycemic control was determined by HbA1C and DPN by the Michigan Neuropathy Screening Instrument (MNSI). Concentric and isometric torque during knee and ankle flexion and extension were assessed by isokinetic dynamometry and torque suitability by principal component analysis (PCA). The identified variables were further used in a cluster analysis (k-means). Stepwise regression was applied to investigate factors associated with HbA1c and MNSI scores. For passive torque analyses, three groups of men (n=49) of similar age were studied, 17 with DM2 without DPN, 15 with DM2 and DPN, and 17 control subjects without DM2. Knee flexion and extension passive torque as well as ankle dorsiflexion and plantar flexion were assessed on an isokinetic dynamometer, followed by passive torque and passive stiffness calculation. The absence of muscular activity during the tests was determined by electromyography (EMG). **Results:** Concentric knee flexion and extension and isometric ankle extension torques characterized 88.59% of the individuals, forming Cluster 1 (n=29 controls) and Cluster 2 (n=59 DM2). HbA1c was associated with lower torque and higher IL-6, and MNSI score with lower torque and higher TNF-α and IL-6. Subjects with DM2 and DPN exhibited greater knee extension passive stiffness (p<0.01) as well as increased passive torque and stiffness during dorsiflexion and plantar flexion (p<0.04) at 5º/s when compared to controls and those with DM2 without DPN. **Conclusion:** Concentric knee and isometric ankle torques discriminated between subjects with and without DM2. However, knee and ankle torque reductions associated with DPN, poor glycemic control and subclinical inflammation were not sufficiently significant to differentiate between DM2 individuals with and without DPN. Individuals with DM2 and DPN present higher knee and ankle stiffness and passive torque in comparison to those with DM2 without DPN and the controls. The mechanical impairments in ankle viscoelastic structures were most evident and more easily assessed at low speeds.

Key-words: diabetes; skeletal muscle; cytokines; lower limb; ankle; knee, joint stiffness

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ELEMENTOS TEXTUAIS

CONTEXTUALIZAÇÃO/PREFÁCIO

Inserção na linha de pesquisa do(a) orientador(a) e do programa

Este trabalho se insere na linha de pesquisa da orientadora: Plasticidade do Músculo Esquelético e Suas Implicações Para a Área de Fisioterapia, e também na linha de Reabilitação Função Motora e Análise Biomecânica do Movimento Humano do Programa de Pós-graduação em Fisioterapia da Universidade Federal de São Carlos.

Parcerias nacionais e internacionais:

- Profª Drª Ângela Merice de Oliveira Leal e Prof. Dr. Henrique Potti-Junior, Departamento de Medicina da Universidade Federal de São Carlos;

- Profª Drª Isabel Camargo Neves Sacco, Departamento de Fisioterapia, Fonoaudiologia e Terapia Ocupacional, Faculdade de Medicina, Universidade de São Paulo;

- Prof. Dr. Fabrício A. Magalhães e Profª. Drª Vanessa L. Araújo, Departamento de Fisioterapia da Universidade Federal de Minas Gerais;

- Prof. Dr. Ricardo Fernandes, Departamento de Engenharia Elétrica da Universidade Federal de São Carlos;

- Prof. Drª Smita Rao, Department of Physical Therapy, Steinhardt School of Culture, Education and Human Development, New York University (NYU).

Estágio de pesquisa nacional e internacional

Foi realizado estágio de pesquisa nacional no Departamento de Fisioterapia da Universidade Federal de Minas Gerais, sob supervisão da Prof.ª Drª. Vanessa L. Araújo. Este estágio foi viabilizado pelo Prof. Dr. Sergio Teixeira Fonseca e teve como objetivo a elaboração do protocolo para a análise do torque e rigidez passiva nos participantes do estudo e também o treinamento do doutorando para essa análise. O grupo do Prof. Dr. Sergio Teixeira Fonseca é uma referência internacional em estudos sobre análise da rigidez passiva

em humanos e este estágio foi muito importante para o aperfeiçoamento do doutorando para realização de estudos sobre este tema.

Foi realizado estágio de pesquisa internacional no Department of Physical Therapy, Steinhardt School of Culture, Education and Human Development, New York University (NYU), com bolsa FAPESP (Processo- 2019/07563-7), sob supervisão da Prof. Drª Smita Rao. Durante o estágio de pesquisa no exterior, o doutorando teve a oportunidade de trabalhar no projeto intitulado "The effects of exercise on individuals with diabetes and Neuropathy" financiado pelo National Institutes of Health (1R01DK114428-01A1, $1,468,323). Este estágio permitiu o aprimoramento profissional do doutorando no atendimento de indivíduos com diabetes e neuropatia periférica, assim como a elaboração de um manuscrito em colaboração com o grupo de trabalho do Brasil e a pesquisadora do exterior.

Originalidade

Este é um estudo original analisando a rigidez e o torque passivo nos movimentos de flexão e extensão do joelho e dorsiflexão e flexão plantar do tornozelo em indivíduos diabetes mellitus tipo 2 (DM2) com e sem neuropatia diabética periférica (NDP), sob diferentes velocidades de movimento (5°/s, 30°/s e 60°/s). Não se observa outros estudos analisando o torque e a rigidez passiva nesta população, separando indivíduos com e sem NDP e confirmando o silêncio muscular por meio da análise do sinal eletromiográfico. Este estudo permite compreender como as alterações do colágeno em indivíduos com DM2, que são bem descritas na literatura, afetam o movimento humano em diferentes velocidades de movimento. Este estudo contribui para explicar a relação entre rigidez aumentada e a manutenção do torque excêntrico em DM2 com e sem NDP, previamente observada em um estudo do nosso laboratório. Para a área da biomecânica clínica, este estudo também é original ao apresentar as séries temporais do torque elástico passivo para os movimentos de flexão e extensão do joelho e tornozelo de DM2 com e sem NDP e controle sem diabetes. Dados sobre o comportamento do torque passivo nestes movimentos podem contribuir para futuros estudos de modelagem biomecânica, permitindo modelos mais realísticos sobre as propriedades mecânicas dos tecidos musculoesqueléticos distais nesta população. Além disso, este trabalho também mostra que o pico de torque isométrico e concêntrico do joelho

e tornozelo podem distinguir a indivíduos com e sem DM2 e que o torque isométrico extensor do tornozelo também pode identificar a presença da NDP.

Contribuição dos resultados da pesquisa para o avanço científico

Os resultados desta pesquisa contribuem para a compreensão das seguintes questões:

- Como a glicação enzimática e acúmulo de agentes avançados de glicação das estruturas do colágeno afeta a resistência elástica do músculo esquelético em humanos;

- Quais movimentos do membro inferior são acometidos pelo aumento da rigidez sob diferentes velocidades de movimento;

- Este estudo também contribui para a compreensão de como a função excêntrica, previamente investigada, pode estar alterada devido ao aumento da rigidez passiva;

- Outro avanço científico foi identificar que o pico de torque concêntrico e isométrico podem ser utilizados como uma variável de rastreio para a DM2 e a NDP.

Relevância social

Este estudo mostra uma maior rigidez passiva em indivíduos com DM2 e NDP em movimentos do joelho e tornozelo. Socialmente, este resultado indica que o aumento da rigidez pode impactar principalmente na qualidade de vida e locomoção da população com DM2 e NDP, pois sabe-se que a rigidez passiva aumentada promove uma resistência durante os movimentos, limitando a realização de atividades de vida diária. Além disso, a redução da mobilidade do tornozelo-pé também leva ao aumento da pressão plantar sendo um esse um fator de risco para ulceras e amputações. Embora a DM2 apresente índices epidêmicos em todo o mundo, as alterações musculoesqueléticas e distúrbios funcionais são pouco reconhecidos nesta população. Este estudo também contribuiu socialmente ao indicar que o

aumento da rigidez musculoesquelética previamente identificado apenas em estudos com animais se manifesta em importantes movimentos dos membros inferiores. Este estudo também traz outra importante contribuição social ao mostrar que o pico de torque concêntrico e isométrico de flexão e extensão do joelho, dorsiflexão e flexão plantar do tornozelo podem distinguir indivíduos DM2 com e sem NDP dos controles sem diabetes. Considerando que a DM2, assim como a NDP se desenvolvem silenciosamente e que o diagnóstico precoce é o principal desafio para o manejo adequado desta doença, este resultado pode contribuir para a triagem precoce da DM2 e da NDP.

Lista de referências de artigos (publicados, submetidos ou em fase de submissão), patentes, eventos/resumos, prêmios, participação em projetos de pesquisa e extensão ou outros produtos desenvolvidos pelo aluno durante o doutorado;

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FERREIRA, JEAN P; ARAÚJO, VANESSA L.; SERRÃO, PAULA R. M. S; PEREA, JULYA P. M. SANTUNE, AFONSO H. A.; POTT-JUNIOR, HENRIQUE; FERNANDES, RICARDO A. S.; ARANHA, GABRIEL D. A. SALVINI, TANIA F. Peripheral neuropathy is related to higher ankle passive torque and stiffness in people with type 2 diabetes Journal: Brazilian Journal of Physical Therapy. Submitted on 2020-09-25.

Link do currículo Lattes e ORCID

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Descrição da tese para o público leigo (máximo 5 linhas)

Este estudo mostra que pessoas com diabetes tipo 2 têm maior rigidez articular no joelho e no tornozelo, e que isso é pior somado com as alterações dos nervos causadas pela diabetes. As alterações da força estão muito ligadas com à diabetes, permitindo a identificação de pessoas com essa doença por meio de medidas da força.

REVISÃO DA LITERATURA

A diabetes mellitus (DM) é uma doença crônica que atingiu índices epidêmicos em todo mundo [1]. O Brasil é o 4º país com maior número de casos, estando atrás apenas da Índia, China e Estados Unidos [1,2]. Os principais tipos são DM tipo 1 (DM1) e DM tipo 2 (DM2), sendo que 90% a 95% dos casos são de DM2 [1,3]. A DM2 é comum em indivíduos adultos sedentários e com sobrepeso [4,5], caracterizada principalmente pela resistência à insulina, que se instala por meio de um mecanismo inflamatório onde o tecido adiposo visceral passa a produzir citocinas inflamatórias como fator de necrose tumoral (TNF-α); interferon gama (INF-γ); interleucina 1 beta (IL-1β) [6]. Essas citocinas ativam a transcrição de proteínas inflamatórias intracelularmente, bloqueando a via de sinalização da insulina [4].

Os principais desafios perante a alta prevalência de DM2 são realizar o diagnóstico precoce e identificar as complicações desta doença, permitindo estratégias clínicas e preventivas tanto para a DM2 quanto suas complicações [7]. A Sociedade Americana de Diabetes [1,8] ressalta a necessidade de se detectar a DM2 precocemente para fins epidemiológicos [1,8], pois isso possibilitaria o manejo preventivo desta doença em grandes massas populacionais. No entanto, a American Diabetes Association (ADA) [7] ressalta a carência de variáveis para monitoramento e controle dos risco para a DM2, e também encoraja pesquisadores a investigarem potenciais variáveis para se monitorar o risco para a DM2 [7], pois, embora existam exames sanguíneos confiáveis para o diagnóstico clínico da DM2 ou da pré-diabetes, essa doença se instala quase sempre sem a manifestação dos sintomas clássicos [7], o que impede o diagnóstico e manejo clínico e preventivo. Neste sentido, um estudo recente sugere a utilização da força como uma variável de rastreio para a DM2 [9], de modo que a perda de força está associada com o pior controle glicêmico [10,11] e também poderia definir um limiar de risco para essa doença [9,11]. No entanto, ainda existe uma limitação para a caracterização de indivíduos com DM2 por meio de medidas de função muscular, pois, não se observa estudos considerando o possível efeito da NDP como uma condição mais crônica que pode estar afetando este limiar. Também não se sabe se há algum grupo ou tipo de contração muscular mais adequados para essa inferência, uma vez que indivíduos DM2 apresentam alterações em diferentes grupos musculares ou articulações [12–15], e que podem estar associadas ao mau controle glicêmico, aumento sérico de citocinas inflamatórias e à NDP.

Segundo a revisão sistemática de Dsouza et al. [16], a miopatia diabética envolve alterações que não foram bem compreendidas e as perdas motoras podem estar presentes em todas as fases desta doença. As alterações musculoesqueléticas que ocorrem na fase precoce da DM2 são explicadas pelo aumento de citocinas pro-inflamatórias, que ativam vias de degradação proteica [17–19] e de apoptose do DNA muscular [20], levando à perda de massa e força muscular. O aumento do estresse oxidativo, associado à hiperglicemia, também acarreta em carbonilação proteica, tornando as estruturas do músculo esquelético envelhecidas e rígidas [4]. Além destes mecanismos, estudos com animais mostram que o mau controle glicêmico leva à glicação não enzimática do colágeno, e associado a isso, há uma alteração dos colágenos tipo III e V nos ligamentos, tornando estas estruturas desidratadas e menos complacentes [21]. Este processo também leva ao acúmulo de agentes avançados de glicação [22,23], tornando a matriz do colágeno mais envelhecida e rígida de forma permanente [23,24]. Assim, seria interessante que as alterações musculoesqueléticas que podem estar diretamente relacionadas à DM2 ou uma condição mais crônica como à presença da NDP sejam consideradas nos estudos sobre a força e a rigidez nesta população.

As primeiras alterações da força ou torque observadas em indivíduos com DM foram associadas à NDP [25–27]. Porém, estudos recentes observaram menor torque concêntrico [28–31] e isométrico [31] e uma manutenção do torque excêntrico [31] em indivíduos DM2 independente da NDP, nos movimentos de flexão e extensão do joelho e dorsiflexão e flexão plantar do tornozelo. As alterações dos torques concêntrico e isométrico, são explicadas pelo aumento sérico de citocinas pró-inflamatórias nesta população [32–36]. No entanto, estudos sugerem que o comportamento sedentário, também conhecido por levar à perda de massa e força muscular por desuso [37] seja uma das principais causas da DM2 [38,39], pois, segundo Hamilton et al., [40], a atividade do músculo esquelético exerce uma importante função endócrina para o controle metabólico e a prevenção da DM2. Embora a possibilidade de se caracterizar a presença da DM2 por meio de medidas de força ou torque [9,11] possa contribuir para a elaboração de estratégias preventivas desta doença, ainda são poucos os estudos neste sentido [9,11] e também seria muito interessante compreender como indivíduos DM2 com e sem NDP se diferenciam em relação a força, nesta caracterização, quando comparados a indivíduos controles sem DM.

A redução dos torques concêntrico e isométrico observadas nesta população [31], representam as alterações dos componentes ativos (geradores de tensão) do sistema

musculoesquelético. No entanto, sabe-se que os componentes passivos elásticos (transmissores de tensão), como fáscias, tendões, ligamentos e cápsula articular também podem ser afetados pela DM2 [14], e principalmente pela hiperglicemia [41–44] que altera a matriz do colágeno do músculo esquelético [21]. Sabe-se que a resistência elástica dessas estruturas é muito importante para a função excêntrica, que é composta pelas forças ativa, proveniente da contração muscular e passiva produzidas pelo alongamento das estruturas [45]. Assim, considerando que estas forças (ativa e passiva) estão diretamente ligadas à função excêntrica, em um estudo prévio [31] apresentamos a hipótese de que a manutenção do torque excêntrico, observada em indivíduos DM2 com e sem NDP estaria associada ao aumento da rigidez passiva nesta população, uma vez que estes indivíduos também apresentaram menor torque concêntrico e isométrico.

Embora se observe estudos com animais mostrando que a DM acarreta em aumento da rigidez [44,46], calcificação dos tendões [47] e alterações do colágeno articular [21], este tema ainda é controverso em estudos com humanos. Apesar de indivíduos DM terem apresentado menor amplitude de movimento (ADM) em relação a indivíduos sem DM, não foi observado diferença em relação à rigidez e ao torque passivo dos flexores plantares, analisado a 60°/s [48]. No entanto, a ADM foi ajustada individualmente para o teste, baseado em uma prévia avaliação de goniometria, o que pode ter afetado o padrão da tensão produzida pelo alongamento das estruturas. Um trabalho posterior observou uma maior rigidez em indivíduos DM, também associada a diminuição da ADM [49]. No entanto, [49] não apresentaram informações sobre a ausência de ativação muscular durante a análise da resistência passiva, o que é fortemente recomendado para se analisar a rigidez passiva [50–53]. Ambos estudos [48,49] não consideram a presença da NDP, que caracteriza uma condição mais crônica desta doença e que pode envolver diferentes alterações musculoesqueléticas [13]. Assim, mesmo havendo estudos com animais indicando um aumento da rigidez, ainda não está claro se a rigidez e o torque passivo estão aumentados em indivíduo DM2 com e sem NDP.

Considerando a importância deste tema para a compreensão da relação entre as alterações da função muscular e da rigidez com a DM2 e a NDP, este estudo apresenta as seguintes perguntas:

- Indivíduos DM2, com e sem PND, apresentam maior rigidez e torque passivos nos movimentos de flexão e extensão do joelho e tornozelo?

- Os torques concêntrico e isométrico, que estão reduzidos nesta população, podem distinguir indivíduos DM2 com e sem NDP de controles sem DM da mesma faixa etária e características antropométricas?

- O controle glicêmico, a presença da NDP e o nível sérico de citocinas pro-inflamatórias estão associadas ao menor torque concêntrico e isométrico de indivíduos DM2?

OBJETIVOS GERAIS DA PESQUISA

Analisar se os torques concêntricos e isométricos podem distinguir indivíduos com DM2 e NDP de indivíduos sem DM com a mesma idade e características antropométricas e também, analisar o torque e a rigidez passiva em indivíduos DM2, com e sem NDP, nos movimentos de flexão e extensão do joelho e do tornozelo, comparados com indivíduos sem DM.

Objetivo específico

- Analisar o torque e a rigidez passiva nos movimentos de flexão e extensão do joelho e dorsiflexão e flexão plantar do tornozelo, em DM2 com e sem NDP, sob diferentes velocidades de movimentos (5°/s, 30°/s e 60°/s).

- Analisar se os picos de torque concêntrico e isométrico do joelho e tornozelo podem diferenciar indivíduos DM2 com e sem NDP e controles sem DM com a mesma idade e características antropométricas;

- Analisar a relação entre os picos de torque concêntrico e isométrico, o percentual de HbA1C, a presença da NDP, citocinas pro-inflamatórias (IL-1β, IL-6 e TNF-α) e as características clínicas dos indivíduos.

Anexo I - Manuscrito 1 – Artigo Submetido ao *Brazilian Journal of Physical Therapy* em 25 de setembro de 2020

Peripheral neuropathy is related to higher ankle passive torque and stiffness in people with type 2 diabetes

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Abstract

The objective of this study is to investigate stiffness and passive torque in individuals with DM2, with and without DPN, during knee and ankle flexion and extension compared to those without diabetes. Our hypothesis is that individuals with DM2, with and without DPN, will present increased stiffness and passive torque in the movements analyzed. **Methods:** Three groups of men (n=49) of similar age were studied, 17 with DM2 without DPN, 15 with DM2 and DPN, and 17 control subjects without DM2. Knee flexion and extension passive torque as well as ankle dorsiflexion and plantar flexion at 5°/s, 30°/s and 60°/s were assessed on an isokinetic dynamometer. The absence of muscular activity during the tests was determined by electromyography (EMG). **Results:** Subjects with DM2 and DPN exhibited greater knee extension passive stiffness ($p<0.01$) as well as increased passive torque and stiffness during dorsiflexion and plantar flexion ($p<0.04$) at 5^{o}/s when compared to controls and those with DM2 without DPN. **Conclusions:** Individuals with DM2 and DPN present higher knee and ankle stiffness and passive torque in comparison to those with DM2 without DPN and the controls. The mechanical impairments in ankle viscoelastic structures were most evident and more easily assessed at low speeds.

Keywords: Biomechanics; joint; collagen alteration; range of motion

BACKGROUND

Type 2 diabetes mellitus (DM2) is associated with connective tissue alterations and physical functionality disorders [1,2] DM2 causes collagen aging, proteoglycan level reduction, increasing stiffness in the ligaments and cartilage [3]. These changes result from the collagen glycation,[4] which, leads to the accumulation of advanced glycation end products (AGEs) [5,6]. The musculoskeletal stiffness heightens the risk of injuries,[7] reduces range of motion (ROM) and causes functional impairment[8,9].

Although it is a relevant topic for affecting the functionality and quality of life, the DM2 effect on joint stiffness is still unclear in humans. The first study[10] found no change in plantar flexor passive torque and passive stiffness in individuals with diabetes mellitus (DM) at 60°/s, compared to controls without DM, despite their exhibiting less ROM. However, the range of motion to access the passive torque was adjusted according to a previous test of goniometry, and it may not have allowed the skeletal muscle stretching to access the maximum passive stiffens. In a subsequent study [11], DM subjects presented a greater passive stiffness during ankle dorsiflexion, also associated with decreased ROM; however, the muscle activation was not controlled during testing which could bias the results [12–15].

Elastic strain energy contributes significantly for total muscle force in activities such as walking [16] and climbing stairs [17,18]. Changes in the joint stiffness in DM individuals also can affect skeletal muscle performance in daily tasks, and reduce the quality of life [16–18]. In both studies that analyzed the stiffness in DM subjects[10,11], the presence of DPN was not considered, although this condition may result in further musculoskeletal impairments. A recent study [19] observed a maintenance of the eccentric torque at 60°/s during knee and ankle sagittal motion while the concentric and isometric torques were altered in DM2 patients with and without DPN than controls without DM. This study hypothesized that increased stiffness is contributing to the eccentric torque maintenance in these subjects. Although DPN was considered, this study results did not clarify the role of DPN in the stiffness and passive torque of distal joints, which remains unknown.

In addition to DM musculoskeletal impairments that might be linked to increased joint stiffness, DPN progressively changes foot-ankle biomechanics during gait,[20–24] represented by the reduced ankle ROM in the sagittal and frontal planes [25,26] and altered muscle dynamics [27–29]. Considering the significant contribution of the elastic strain energy for the distal joint torques during gait [16–18] and that the DPN affects mainly the distal extremities,[20–24] investigating tissue stiffness and passive torque in distal joints would

improve a patient's dynamic characterization for tailoring preventive and treatment actions. The global consensus is that individuals with DM and DPN should protect their feet at all times to minimize the risk of tissue damage [30]. Because of this principle, protective strategies are preferred over therapeutic exercises to regain the lost functionality usually linked to DM and DPN progression [30]. Restricted joint movement as a strategy implemented in clinical settings changes the foot rollover, which is the main cause of alterations in plantar pressure that are well-known risk factors for foot ulcer development. Therefore, a better understanding of the tissue and joint stiffness and passive torque would contribute to the to the next recommendations in the international guidelines [31] to further discussion surrounding the need to include a regime of therapeutic exercises to preserve and improve the functionality of the individual's foot-ankle, since the elastic strain energy is an important component of the skeletal muscle function and can be improved through stretching and strengthening exercises. In addition, data on the passive stiffness and passive torque in DM2 and DPN subjects would potentially contribute to biomechanical modeling studies aimed at calculating joint and muscle forces in these individuals [22,32]. Whereas the passive elastic resistance changes according the speed of motion [33], it would be interesting to analyze the passive torque at 5°/s (minimal velocity of the equipment), avoiding reflex muscle activation [34] and also at 30°/s and at 60°/s for being similar to gait speeds of motion [10,35,36].

The present study aimed to investigate passive stiffness and torque in individuals with DM2, with and without DPN, during knee and ankle flexion and extension compared to those without diabetes at 5°/s, 30°/s and 60°/s. Our hypotheses are (1) that individuals with DM2, with and without DPN, will exhibit greater passive stiffness and passive torque than controls, and (2) that passive stiffness and passive torque will be higher in those with DPN when compared to controls and individuals with DM2 for the movements analyzed.

METHODS

Participants

Given that stiffness is more marked in men than women [37], indicating the importance of comparing groups also matched for sex, only male volunteers aged between 18 and 56 years were recruited. The study was conducted in line with the human research guidelines and regulations (National Health Council Resolution 466 of 2012; Laws 11.794, 8.080 and 8.142) and approved by the institutional Research Ethics Committee (protocol 1.930.043).

Inclusion and exclusion criteria

The control group consisted of subjects without DM, the DM2 group of those with DM2 and no DPN, and the DPN group of individuals with DM2 and DPN. Those with self-reported osteoarticular diseases (rheumatoid arthritis, arthrosis, history of fractures, herniated discs, orthopedic leg surgery, etc.), prediabetes, non-diabetic neuropathies, history of peripheral artery disease and body mass index (BMI) >35 kg/cm² were excluded.

A total of 75 individuals were interviewed for eligibility, 51 of whom met the criteria and were included for analysis, distributed into three groups: control (n=17); DM2 (n= 17) and DPN (n=17) (Figure 1). The presence of DM2 was determined by an endocrinologist in accordance with the American Diabetes Association criteria [38], using the glycated hemoglobin test (HbA1c) and considering DM2 $\geq$ 6.1%.

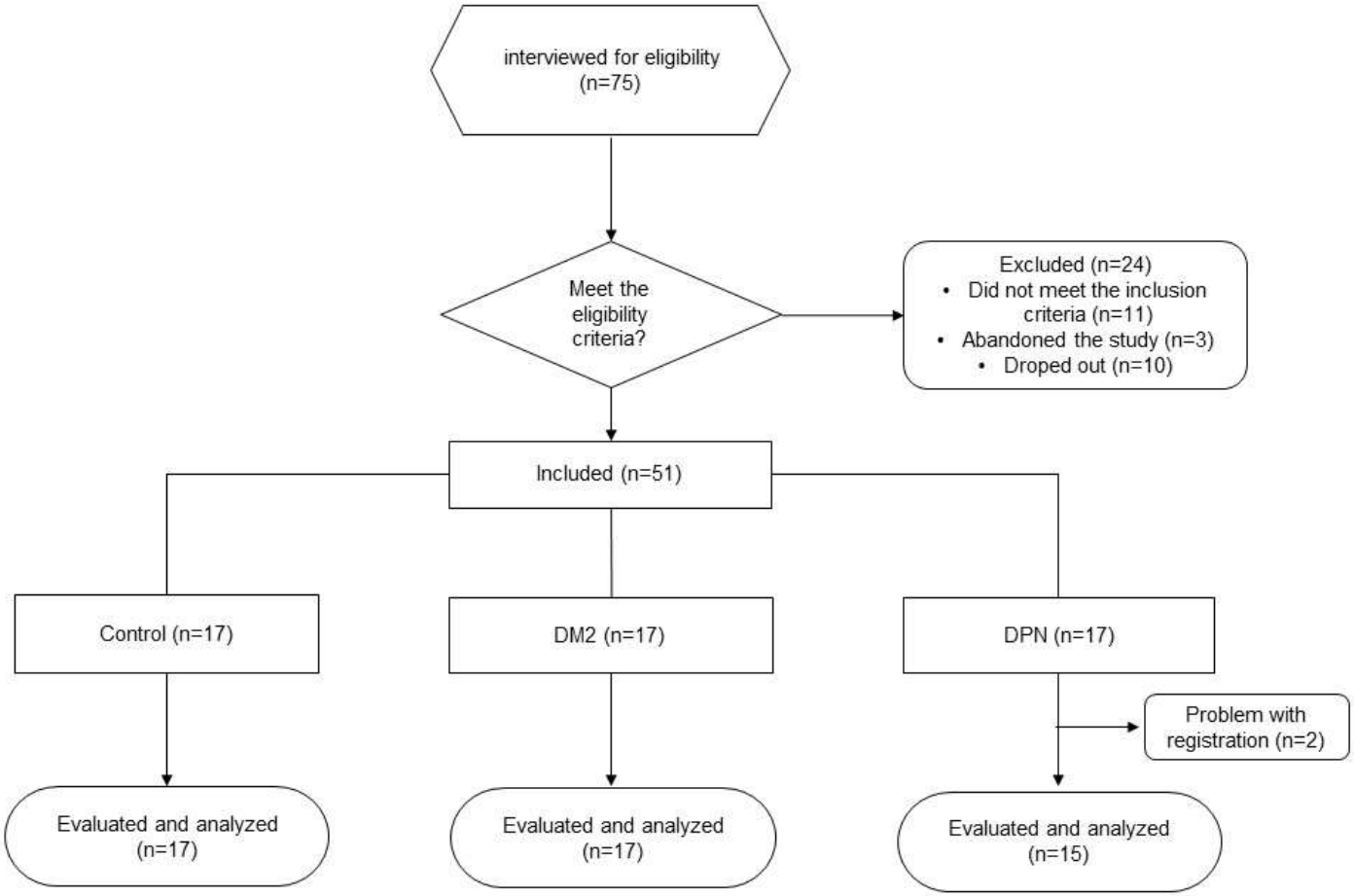

Figure 1. Flowchart of the study design
Control = control group; DM2= Type 2 diabetes; DPN= Type 2 diabetes with peripheral neuropathy.

The presence or not of DPN was based on highly reliable and reproducible assessments [39]. Symptoms of DPN were evaluated via the Michigan Neuropathy Screening Instrument (MNSI), validated for Brazilian Portuguese [40]. Tactile (10 g monofilament on the plantar surface of the hallux and 1st, 3rd and 5th metatarsal heads) and vibratory sensitivity (128Hz vibration in the medial region of the hallux interphalangeal joint) were evaluated because these sensory losses are most typical of DPN [41,42]. An artificial intelligence system based on fuzzy logic was used to determine the DPN severity [27,43]. A score $\geq$ 2 was considered DPN [44]. This classification model present a high accuracy receiver operating characteristic (ROC) curve= 0.91 and strong correlation with specialist opinion (Pearson's correlation coefficient = 0.94) [43].

Given that exercise or physical activity can improve strength and flexibility, all participants answered the habitual physical activity questionnaire (Baecke) validated for Brazilian Portuguese [45]. It allows access the levels of habitual physical activity within the past 12 months, including: 1) occupational physical activities (8 questions); 2) physical exercises in leisure (PEL) (4 questions); and 3) leisure and locomotion activities (LLA) (4 questions). The scores are calculated as described: Modality 1 (Intensity x Time x Proportion) + Modality 2 (Intensity x Time x Proportion). The remaining questions assess the exercise level during leisure hours (e.g. "during leisure hours, I practice sports or physical exercises"). The answers are scored on a scale from 1 (never) to 5 (very often) and the total score is determined by PEL + LLA [45].

The groups were not different in terms of age, anthropometric characteristics and occupational physical activity, sports or leisure-time exercise (Table 1), and the diabetic groups did not differ in relation to clinical characteristics (Table 1).

Table 1. Clinical and demographic characteristics of the participants

	Control (N=17)	DM2 (N=17)	DPN (N=15)	ANOVA
Age (years)	53.62 (8.74)	59.35 (6.55)	57.02 (7.25)	*P* =0.08; F=2.54
Time since diagnosis (years)	0 (0.00).	12.06 (7.87) *	11.13 (6.54) *	*P* <0.01; F=21.06
Body mass (kg)	85.47 (16.65)	81.49 (12.07)	91.83 (16.33)	*P* =0.16; F=0.88
Body Mass Index (kg/m²)	26.4 (4.1)	27.8 (3.1)	30.7 (5.0)	*P* =0.32; F=1.15
Height (m)	1.73 (0.05)	1.69 (0.05)	1.73 (0.06)	*P* =0.06; F=2.92
HbA1C (%)	5.3 (0.3)	8.0 (2.5) *	8.1 (1.9) *	*P* <0.01; F=12.32
DPN severity fuzzy score	0.64 (0.02)	1.28 (0.71)	4.35 (2.63) *†	*P* <0.01; F=26.24
Michigan questionnaire score	0.47 (0.62)	2.52 (1.97) *	6.93 (2.31) *†	*P* <0.01; F=55.05
Vibration sensitivity right present/ reduced/ absent (number of patients)	17/0/0	17/0/0	9/2/4	-
Vibration sensitivity left present/ reduced/ absent (number of patients)	17/0/0	17/0/0	11/1/3	-
OPA	6.39 (1.50)	5.83 (2.51)	5.94 (2.23)	*P*=0.72; F=0.32
SA	1.32 (2.59)	1.32 (2.13)	0.55 (1.20)	*P*=0.52; F=0.66
SSA	0.91 (2.18)	0.94 (0.58)	0.0 (0.0)	*P*=0.14; F=2.04
TISA	2.44 (4.49)	1.51 (2.24)	0.55 (1.20)	*P*=0.24; F=1.46
PELS	4.95 (1.03)	4.72 (1.12)	4.44 (1.26)	*P*=0.50; F=0.70

Data were expressed as mean (standard deviation). *=*P*<0.05 in relation to controls. †=*P*<0.05 in relation to DM2. m= meters, kg = kilograms. Control = control group; DM2 = Type 2 diabetes without diabetic peripheral neuropathy; DPN = Type 2 diabetes with diabetic peripheral neuropathy. Physical activity indexes: OPA (occupational physical activity); SA (sports activity); SSA (second sports activity); TISA (total index of sports activities); PELS (physical exercises in leisure score).

Passive torque assessment protocol

Instruments

An isokinetic dynamometer (Biodex Multi-joint System 3, USA) was used to evaluate passive torque during knee and ankle flexion and extension at: 5°/s, recommended for evaluating passive torque [46], and 30 and 60°/s, which are common speeds in gait [35,47]. The order of analysis for joints and velocities was randomized [48].

In order to ensure the electrical silence of muscles during movement, EMG of knee flexors (semitendinosus and biceps femoris) and extensors (vastus lateralis, rectus femoris and vastus medialis) as well as ankle dorsiflexors (tibialis anterior) and plantar flexors (soleus and medial gastrocnemius) were collected by a Trigno™ Mobile EMG system (Delsys Inc., USA). The electrodes were attached to the skin following all SENIAM (Surface Electromyography for the Non-Invasive Assessment of Muscles) recommendations [49,50].

Positioning participants on the isokinetic dynamometer

Passive knee torque was evaluated with participants sitting on the dynamometer, guaranteeing electrodes clearance over the seat (Figure 2 A and B). The hips were stabilized at approximately 85° of flexion. Knee flexion at 90º was considered the starting position (position 0). The axis of the dynamometer was aligned with the lateral epicondyle of the femur and the attachment fixed to the distal third of the leg.

For ankle passive torque assessment, participants were seated on the equipment with their hips and trunk stabilized at 70° of flexion. The leg was supported and maintained with the knee at 30° flexion, and the foot to a platform in a neutral position (i.e. position 0, perpendicular to the shank). The axis of the dynamometer was aligned with the lateral malleolus. Participants were asked to wear shoes to better accommodate their feet on the platform.

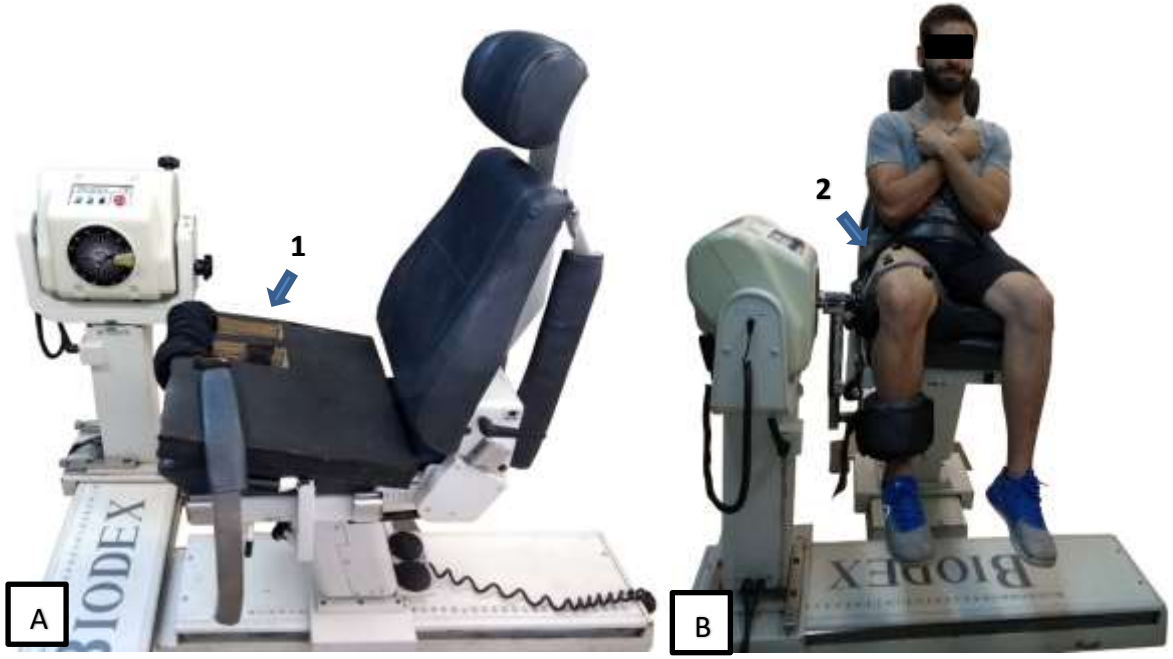

Figure 2. A - Adapted seat to assess surface electromyography of the hamstring muscles in the sitting position on an isokinetic dynamometer; 1 - compartment to accommodate the sensors. B – Participant seated on the adapted seat to accommodate the electromyographic sensors during passive torque assessment; 2 - Electromyographic sensors accommodated on the adaptaded seat.

EMG assessment - checking the absence of muscle activation

EMG activity was measured at rest, before testing and during the tests. After each repetition in the passive torque tests, the EMG signal was processed in real time using a code of MATLAB software (version 7.0.1, Matworks, USA) to detect muscle activation. EMG signals were filtered using a 4th order band-pass Butterworth filter (zero-lag,10Hz to 500Hz) [51]. The signal during passive torque testing was compared to that obtained at rest in 100ms intervals. Muscle activity was deemed present when the mean of the signal was $\geq$ 2 standard deviations of the resting signal [15]. In the event of muscle activation, that repetition was disregarded and repeated [46,51] until three valid repetitions were obtained.

Processing passive torque

The passive torques and angular displacement were processed using MATLAB software. The signal was filtered using a 4th order band-pass Butterworth filter (zero-lag), with a 1.25Hz cutoff frequency. The torque produced by the weight of the knee and ankle attachments was subtracted from total knee and ankle torque, respectively. Knee torque was calculated by subtracting the torques generated by shank and foot weight from total torque, and ankle torque by subtracting the torque generated by foot weight from total torque. The following calculation determined the torque of the shank and foot weight:

$$N.m = (Bw \times g) \times \%\,Bw \times m$$

where, N.m= newton meters; Bw= body weight; g = gravity; % Bw= percentage of the limb weight in relation to total body weight; m= Linear distance in meters between the center of mass and joint axis.

The proportion of the limb's weight in relation to total body is 4.65% for the shank and 1.45% for the foot. The linear distance between the center of mass and the joint axis was measured as proposed by Dempster [52].

A time series of passive torque *versus* motion degrees was generated from the position where torque was equals 0 (N.m) (angle at which the elastic forces of agonist and antagonist muscles are neutral) to the end of movement. The time series are presented as supplementary material for a qualitative analysis. The peak of passive torque, total torque (mean torque produced over ROM) and total passive stiffness were calculated by the slope of the torque curve *versus* angular displacement, as follows:

$$\frac{\Delta\, N.m}{\Delta\, JM}$$

where, Δ= variation; N.m= newton meters; JM= joint motion in degrees.

For all variables the intra-rater reliability was obtained for 7 control individuals. The intraclass correlation coefficients (ICC [2,1]) ranged between moderate (0.4-0.75) and excellent (>0.75). Minimum value observed (ICC= 0.52; SEM= 0.54) and maximum (ICC= 0.99; SEM≤ 0.01) (Table 1).

Table 1. Intraclass correlation coefficient ($ICC_{1.2}$) and standard error of measurement (SEM) for the knee and ankle passive torque and stiffness

Joint motion	Joint speed (degree per second)	Mean of total torque (N-m)	Peak torque (N-m)	Mean of total joint stiffness (N-m x degree)
Knee flexion	5°/s	ICC= 0.89; SEM= 0.39	ICC= 0.88; SEM= 1.52	ICC= 0.97; SEM= 0.01
	30°/s	ICC= 0.65; SEM= 0.75	ICC= 0.92; SEM= 1.07	ICC= 0.99; SEM≤ 0.01
	60°/s	ICC= 0.88; SEM= 0.61	ICC= 0.97; SEM= 0.67	ICC= 0.99; SEM≤ 0.01
Knee extension	5°/s	ICC= 0.88; SEM= 0.25	ICC= 0.89; SEM= 1.56	ICC= 0.45; SEM= 0.02
	30°/s	ICC= 0.57; SEM= 0.51	ICC= 0.96; SEM= 0.55	ICC= 0.99; SEM= 0.05
	60°/s	ICC= 0.63; SEM= 0.69	ICC= 0.93; SEM= 0.83	ICC= 0.88; SEM= 0.01
Ankle dorsiflexion	5°/s	ICC= 0.94; SEM= 0.06	ICC= 0.56; SEM= 0.65	ICC= 0.96; SEM≤ 0.01
	30°/s	ICC= 0.63; SEM= 0.10	ICC= 0.87; SEM= 0.77	ICC= 0.95; SEM= 0.01
	60°/s	ICC= 0.58; SEM= 0.44	ICC= 0.77; SEM= 0.73	ICC= 0.90; SEM= 0.01
Ankle plantar flexion	5°/s	ICC= 0.53; SEM= 0.30	ICC= 0.54; SEM= 0.86	ICC= 0.93; SEM≤ 0.01
	30°/s	ICC= 0.69; SEM= 0.59	ICC= 0.59; SEM= 0.57	ICC= 0.56; SEM= 0.01
	60°/s	ICC= 0.52; SEM= 0.54	ICC= 0.85; SEM= 1.67	ICC= 0.99; SEM= 0.13

ICC value: poor (<0.4); moderate (0.4-0.75); excellent (>0.75). The confidence interval adopted was 95%.

Statistical Analysis

Intergroup differences for clinical and demographic characteristics of the participants and knee and passive torque and stiffness were compared by one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for multiple comparisons. Statistical significance was assessed in a two-tailed test (alpha= 0 Statistical power (1 - β) with a medium effect size was 0. 76..05). All analyses were conducted using

SPSS version 22 (IBM, Somers, USA) and R version 3.5.3 (The R Foundation for Statistical Computing, Vienna, Austria).

Results

The statistical power (1 - β) observed between comparisons with a medium effect size was 0.76. For knee extension, individuals with DPN and DM2 showed greater mean total passive stiffness at 60°/s when compared to controls ($p \leq 0.01$), although with no intergroup differences for mean and peak torque at 5°/s and 30°/s (Table 2). For knee flexion, there was no difference between groups (Table 2).

Table 2. Knee passive torque and stiffness

	Control (N=17)			DM2 (N=17)			DPN (N=15)		
	5°/s	30°/s	60°/s	5°/s	30°/s	60°/s	5°/s	30°/s	60°/s
Knee flexion									
0 N.m angle (degree)	38.93 (12.81)	40.34 (12.91)	35.91 (10.43)	42.03 (13.86)	39.93 (20.25)	43.80 (15.88)	36.80 (10.40)	41.53 (12.44)	46.60 (14.55)
Mean of total torque (N-m)	1.36 (1.38)	1.35 (1.94)	1.15 (1.31)	0.98 (0.75)	1.14 (0.72)	1.15 (1.19)	1.41 (1.45)	1.70 (2.00)	1.72 (1.97)
Peak torque (N.m)	5.32 (1.63)	6.06 (1.72)	6.60 (1.04)	5.27 (1.81)	5.30 (2.28)	6.25 (1.49)	5.89 (2.21)	7.10 (2.34)	7.42 (1.78)
Mean of total joint stiffness	0.05 (0.02)	0.14 (0.05)	0.11 (0.05)	0.11 (0.18)	0.13 (0.07)	0.14 (0.07)	0.06 (0.05)	0.13 (0.06)	0.11 (0.06)
Knee extension									
0 N.m angle (degree)	48.26 (18.46)	58.78 (16.68)	57.43 (11.24)	50.60 (19.56)	58.47 (20.12)	56.93 (20.33)	54.54 (12.41)	62.16 (16.26)	67.14 (17.39)
Mean of total torque (N.m)	1.25 (1.02)	1.38 (1.32)	0.85 (0.56)	1.27 (1.16)	1.11 (0.83)	1.45 (1.15)	1.37 (1.41)	1.63 (1.51)	1.87 (1.41)
Peak torque (N-m)	4.65 (1.65)	4.37 (2.42)	4.58 (1.07)	4.99 (2.01)	4.19 (1.60)	5.00 (5.07)	5.20 (2.28)	4.63 (2.00)	4.56 (2.39)
Mean of total joint stiffness	0.08 (0.03)	0.09 (0.05)	0.05 (0.03)	0.11 (0.04)	0.36 (1.07)	0.14 (0.16)*	0.09 (0.05)	0.08 (0.03)	0.15 (0.14)*

Data expressed as mean (standard deviation). *=$P<0.05$ in relation to controls. N.m= newton meters. Control = control group; DM2 = Type 2 diabetes without diabetic peripheral neuropathy; DPN = Type 2 diabetes with diabetic peripheral neuropathy.

DPN participants exhibited higher ankle plantar flexion peak torque at 5°/s compared to controls (p=0.02) and DM2 ($p\leq$0.01). DPN group presented greater total passive stiffness than controls ($p\leq$0.01) (Table 3). Ankle dorsiflexion peak torque at 5°/s was higher in DPN than the control (p=0.04) and DM2 ($p\leq$0.01). Mean total torque at 5°/s was higher in DPN than control ($p\leq$0.01) and DM2 ($p\leq$0.01). Additionally, DPN presented higher torque at 30°/s than controls ($p\leq$0.01) and greater passive stiffness at 60°/s than DM2 (p=0.03) (Table 3).

Table 3. Ankle passive torque and stiffness

	Control (N=17)			**DM2 (N=17)**			**DPN (N=15)**		
	5°/s	30°/s	60°/s	5°/s	30°/s	60°/s	5°/s	30°/s	60°/s
Ankle dorsiflexion									
0 N.m angle (degree)	98.14 (5.98)	86.25 (20.70)	10.91 (3.05)	100.68 (5.16)	88.47 (20.12)	18.52 (24.07)	99.25 (6.32)	82.16 (16.26)	10.07 (4.07)
Mean of total torque (N.m)	0.49 (0.33)	0.96 (0.77)	1.25 (0.69)	0.45 (0.41)	0.50 (0.36)	1.22 (0.49)	0.93 (0.52)* †	0.73 (0.44)	1.27 (0.95)
Peak torque (N.m)	6.24 (1.62)	6.40 (1.61)	6.68 (1.03)	6.05 (1.48)	5.90 (1.42)	6.33 (1.19)	7.66 (1.57)* †	7.29 (1.01)†	7.40 (1.85)
Mean of total joint stiffness	0.18 (0.09)	0.21 (0.06)	0.23 (0.06)	0.21 (0.06)	0.23 (0.06)	0.26 (0.06)	0.25 (0.08)	0.26 (0.06)	0.28 (0.06)†
Ankle plantarflexion									
0 N.m angle (degree)	92.04 (5.18)	86.25 (20.70)	20.05 (3.08)	92.38 (3.46)	85.42 (13.25)	20.35 (2.43)	92.52 (5.10)	88.83 (5.27)	19.48 (3.62)
Mean of total torque (N-m)	1.04 (0.62)	0.98 (0.50)	0.83 (0.58)	0.98 (0.48)	0.61 (0.40)	0.52 (0.36)	1.06 (0.86)	0.82 (0.72)	1.06 (0.74)
Peak torque (N.m)	7.24 (1.07)	5.28 (1.20)	5.43 (1.31)	7.03 (0.93)	5.11 (1.36)	5.11 (1.17)	8.49 (1.77)* †	6.06 (1.45)	6.27 (1.67)
Mean of total joint stiffness	0.02 (0.02)	0.12 (0.15)	0.11 (0.05)	0.03 (0.01)	0.08 (0.05)	0.11 (0.04)	0.04 (0.01)*	0.08 (0.04)	0.11 (0.03)

Data expressed as mean (standard deviation). *=P<0.05 in relation to controls. †=P<0.05 in relation to DM2. N.m= newton meters. Control = control group; DM2 = Type 2 diabetes without diabetic peripheral neuropathy; DPN = Type 2 diabetes with diabetic peripheral neuropathy.

Discussion

Our results indicate that people with DM2 and DPN exhibit greater passive torque and passive stiffness than subjects without DM and those with DM2 without DPN for dorsiflexion and plantar flexion at 5°/s. This result helps explaining the functional impairment usually described in the literature in individuals with DM2 and DPN [20–24], since increased passive stiffness is known to alter force transmission through the passive musculoskeletal system, reducing muscle strength and power [2]. Clinically, increased passive stiffness also compromises function in activities of daily living [2] and reduces gait speed [2,9].

The passive torque and passive stiffness unchanged between DM2 without DPN and controls indicates that DPN status was a determining factor for increased passive stiffness. Rao et al., (2006) reported greater passive stiffness in dorsiflexion in participants with DM, but our study is the first to investigate whether DPN, which characterizes a more chronic condition of the DM2, contributes to altering passive stiffness. Although time since DM2 diagnosis was the same between DM groups, the presence of DPN was a key factor for this difference. Thus, while the relationship between DPN and time since diagnosis is not necessarily direct, passive stiffness may be directly linked to neuromotor impairments related to DPN and glycemic control. A possible explanation for the high passive torque and passive stiffness observed only in the DPN group in our study may be the longer hyperglycemic period in these individuals as DPN develops [41], meaning that non-enzymatic glycation of collagen likely has a greater effect [1] as DNP develops. This process alters type III and V collagen in the ligaments, increasing their passive stiffness [3]. DM also results in the progressive and irreversible accumulation [1,53] of AGEs in the collagen matrix of the musculoskeletal system [5,6], raising tissue stiffness. Our results suggest that these structures may become even stiffer with the onset of DPN.

Interestingly, the most noteworthy change in torque occurred in ankle motion at 5°/s. The viscoelastic properties of passive tissue are likely significantly changed in this population, which in turn impairs its flexibility. This compromises performance as well as mechanical and metabolic efficiency during activities of daily living, especially those that require greater ranges of motion, such as going up and down stairs at home, in public settings or on buses [2]. Although it was hypothesized that DM2 individuals with and without DPN would exhibit increased passive stiffness and passive torque at all velocities

studied, it did not occur at faster speeds. It is known that while the passive stiffness of the viscous component increases linearly with speed [54], the elastic component passive stiffness remains unchanged [55]. Therefore, a possible explanation for the increased passive stiffness at slow speeds (5°/s) is that those with DM2 and DPN exhibit greater stiffness of elastic component than of the viscous component. The DM2 with PND also showed a higher mean of total passive stiffness for knee extension at 60 °/s, a higher peak of passive torque at 30°/s, and greater passive stiffness at 60°/s for dorsiflexion, compared to control subjects. This result suggests that although the differences at 30°/s and 60°/s were more discreet, stiffness can alter functionality in faster movements.

A qualitative time series analysis demonstrated greater passive torque in the DM2 and DPN groups across the entire movement, peaking at the end, at the largest ROM. Participants with DM exhibited an intermediate pattern compared to DPN and controls. Nevertheless, passive torque remained unchanged between DM2 and DPN throughout ROM in faster velocities. The increased passive stiffness was more easily identified in DM2 subjects at 5°/s. The times series presented in the supplementary material for knee and ankle passive torque at different velocities will contribute to biomechanical modeling studies aimed at calculating joint and muscle forces in subjects with DM2 with and without DPN [22,32], since research depends on in vivo values to implement models that are biomechanically similar to real individuals.

With respect to the lack of difference in passive stiffness and passive torque in almost all knee movements among those with DM2 and no DPN, atrophy and changes in passive structures due to AGE accumulation are likely more accentuated in the distal region of the limbs, as occurs with sensory and motor alterations [20–22].

Motor dysfunction and tissue changes are observed in the early stages of DM and DPN [30,44,56] as decline in motor conduction velocity [57], changes in muscle activity during gait [27] and isometric force [58]. Biopsy [59,60] and muscle fiber conduction velocity [57] studies demonstrated preferential loss of type I fibers in individuals with DM with or without DPN, which was corroborated in experimental animal models [61]. Hendersen et al. (2020) [62] showed that DPN primarily affects intrinsic before extrinsic muscles, with patients showing a decrease in the size of four intrinsic muscles and one extrinsic foot muscle. Changes in muscle function significantly influence the quality and control of locomotion, affecting the absorption and transmission of forces during foot rollover. Given this complex scenario involving patients with DM2 with and without DPN, it can be

concluded that all these neuromuscular factors combined compromise homogeneous plantar pressure distribution, which is a well-known risk factor for foot ulcers. Thus, investigating passive torque and joint passive stiffness in DM individuals, in addition to the more common assessment of muscle activation, may reinforce the need for preventive measures to delay or prevent musculoskeletal impairments related to DM and DPN progression. To date, most biomechanical interventions have been based on using structured and cushioned shoes for all diabetes patients, regardless of their musculoskeletal conditions. However, preventive therapeutic interventions involving active exercise, stretching and joint mobility are highly recommended to delay or prevent the tissue passive stiffness complications investigated here, thereby reducing the impact of this disease on quality of life [63].

This study also has a limitation that should be considered. The cross-sectional design, which precludes establishing an association of causality for the effect of DPN on passive stiffness.

Conclusion

Individuals with DM2 and DPN exhibited greater knee and ankle passive stiffness and passive torque than controls and those with DM2 and no DPN. The mechanical impairments in ankle viscoelastic structures were most evident and more easily assessed at low speeds. The behavior of knee and ankle passive torque in individuals with DM2 with and without DPN can contribute to the biomechanics field making it possible to more realistically model the mechanical properties of distal musculoskeletal tissue in this population. The investigation of passive torque and joint passive stiffness in DM individuals, in addition to the more common assessment of muscle activation, may reinforce the need for preventive measures to delay or prevent musculoskeletal impairments related to DM and DPN progression.

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Authors contributions

T.F.S is the guarantor of this study and, as such, had full access to all the data and takes responsibility for its integrity and the accuracy of data analysis. J.P.F., T.F.S., V.L.A, P.R.M.S. and I.C.N.S. were responsible for the study design, data analysis and interpretation and the literature review. A.M.O.L. and J.P.M.P. contributed to developing the study design and data interpretation. J.P.F., A.H.A.S and J.P.M.P. were responsible for data collection and the reliability of measurements, G. D. A. A., J.P.F and V.L.A for data processing, and H. P. J. and R. A. S. F. for data processing and time series analyses. All the authors contributed to the manuscript.

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Time series presentation

The time series showed similar intergroup passive torque curves for knee flexion and extension at 5°/s and 30°/s. For knee flexion at 60°/s, the slope was steeper at the end of ROM in the DPN group when compared to controls and the DM2, but with no intergroup difference between curves for extension at 60°/s (Figure 3). With respect to the ankle, the DPN exhibited greater torque throughout ROM for almost all the movements analyzed at 5°/s and a steeper slope in the final 10° of motion. However, there was no difference at 60°/s, as occurred at the slower speeds. This change in pattern suggests that although individuals with DPN show high stiffness throughout ROM, it is greatest at the end of movement. The DM2 group displayed intermediate behavior in relation to controls and the DPN for almost all the movements, with higher torque than controls and lower than the DPN throughout joint movement (Figures 3 and 4).

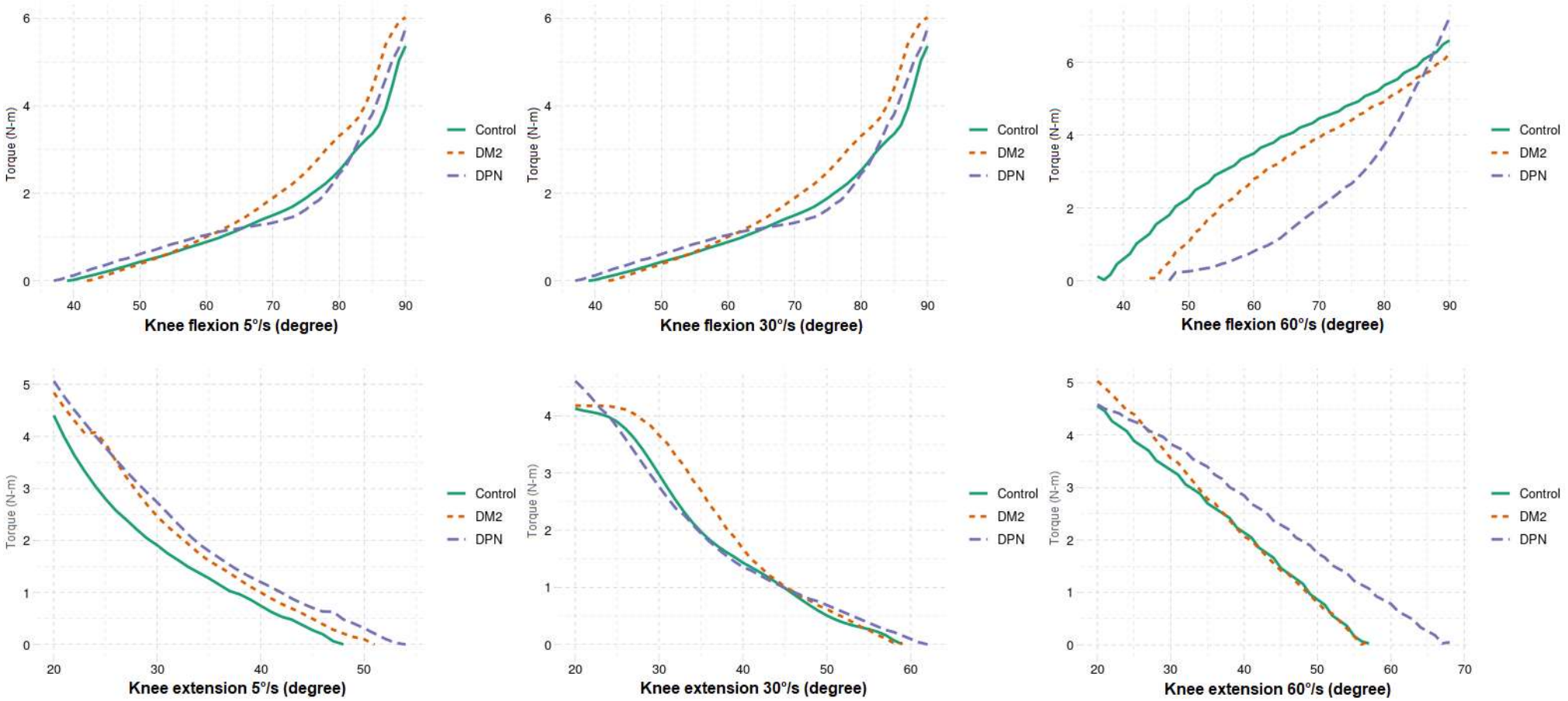

Figure 3. Passive torque time series for knee flexion and extension at 5°/s, 30°/s and 60°/s. N.m= newton meters. Control = control group; DM2 = Type 2 diabetes without diabetic peripheral neuropathy; DPN = Type 2 diabetes with diabetic peripheral neuropathy.

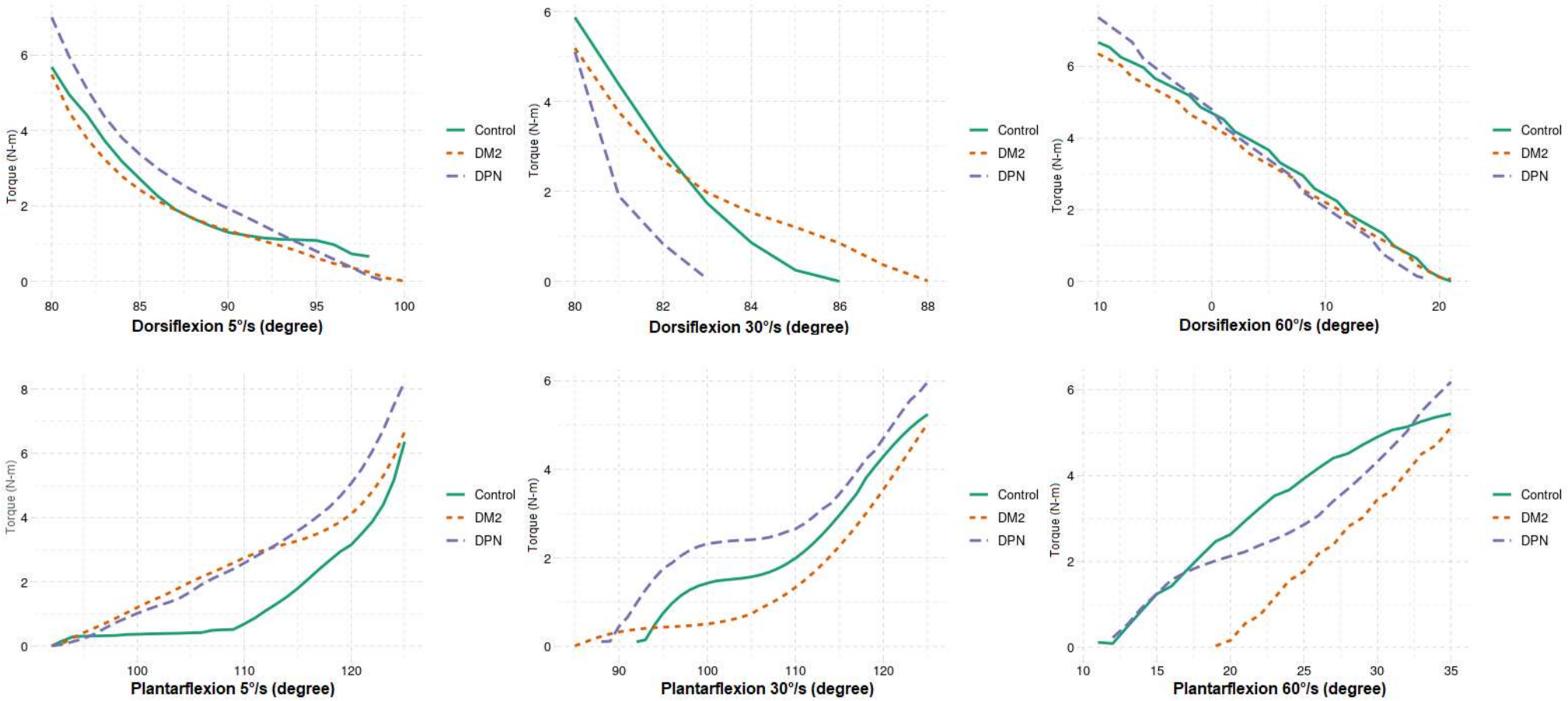

Figure 4. Passive torque time series for ankle dorsiflexion and plantarflexion at 5°/s, 30°/s and 60°/s. N.m= newton meters. Control = control group; DM2 = Type 2 diabetes without diabetic peripheral neuropathy; DPN = Type 2 diabetes with diabetic peripheral neuropathy.

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Anexo II - Manuscrito 2 – Artigo Submetido ao *Journal of Diabetes Research* em 28 de Maio de 2020.

Cluster analysis of joint torque in individuals with and without diabetes and neuropathy

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Abstract

Objectives: Investigate whether knee and ankle torques can differentiate between individuals with and without type 2 diabetes (DM2) and with and without neuropathy (DPN). It was also investigated the relationships between concentric and isometric torques and glycemic control, DPN, proinflammatory cytokines and clinical characteristics to understand which factors are associated with the lower strength. **Methods:** Of the 88 participants, 29 were controls, 59 with DM2 (23 with and 36 without DPN). Glycemic control was determined by HbA1C and DPN by a system based in the Michigan Neuropathy Screening Instrument (MNSI). Concentric and isometric torque during knee and ankle flexion and extension were assessed by isokinetic dynamometry and torque by principal component analysis (PCA). The variables indicated by a PCA were used for further cluster analysis. Multiple linear regression was applied to investigate factors associated with TNF-α, IL1-β, IL-6, HbA1c and MNSI scores. **Results:** Concentric knee flexion and extension and isometric ankle extension torques explained 88.59% of data variation, forming Cluster 1 (n=29 controls) and Cluster 2 (n=59 DM2). HbA1c was associated with lower torque and higher IL-6, and MNSI score with lower torque and higher TNF-α and IL-6. **Conclusion:** Concentric knee and isometric ankle torques discriminated between subjects with and without DM2. However, knee and ankle torque reductions associated with DPN, poor glycemic control and subclinical inflammation were not sufficiently significant to differentiate between DM2 individuals with and without DPN.

Keywords: diabetes diagnosis; diabetes prevention; skeletal muscle; cytokines; lower limb; ankle; knee

INTRODUCTION

Studies show that muscle strength reduction is strongly linked to type 2 diabetes mellitus (DM2) [1,2], making it a variable capable of predicting [3] the presence of this disease.

Investigating alternatives for early DM2 screening is in line with the interests of global organizations that have highlighted difficulties in the early detection [4,5] and management [6] of this disease. Physical activity, essential to maintaining muscle strength [7], is strongly linked to reduced risk of DM2. Meta-analyses [8,9] have shown that sedentary behavior increases the relative risk of DM2 by 112% and that leg muscle activity is important in controlling metabolic syndrome and lowering this risk, due to the number of oxidative fibers [10].

Although these studies indicate that reduced strength is associated with DM2 [1–3], they do not consider the presence of diabetic peripheral neuropathy (DPN), which causes sensorimotor changes in these individuals [11]. Analyzing the presence of NDP will be very important to understand whether NDP contributes or not to this association.

Increased subclinical inflammation [12] results in protein degradation and apoptosis of muscular DNA [13], which is also associated with decreased concentric [14,15] and isometric torque [15] of knee and ankle joints in individuals with DM2 with and without DPN [14–16]. A study found that the decline in muscle strength in this population is greater in the lower than upper limbs [17]. Despite the fact that strength may be influenced by the presence of DPN [18–20] and by higher concentration of proinflammatory cytokines, there is still a gap in the literature about the association between these factors.

The present study aimed to investigate whether knee and ankle concentric and isometric torques can differentiate between individuals with and without DM2 and with and without DPN. In addition, it was also aimed to analyze the relationships between concentric and isometric torques and glycemic control (HbA1C), DPN presence, proinflammatory cytokines levels (IL-1β, IL-6 and, TNF-α), and clinical characteristics were also investigated. Given that torque values are reduced in DM2 subjects[14–16], our hypotheses are that torque will be able to differentiate between individuals with and without DM2, and that glycemic control and the presence of DPN will be associated with lower torque and increased serum

proinflammatory cytokine levels.

Methods

This is a cross-sectional observational study and it was conducted in accordance with the Guidelines and Regulations for Research Involving Human Beings (National Health Council Resolution 196/1996 and 466 of 2012) (Law 11.794, no. 8.080 and 8.142) and approved by the institutional Research Ethics Committee (protocol no. 808.264).

Participants

The minimum sample size was calculated based on the recommendations by Dalmaijer et al. (2020)[21], who suggested that a sufficient statistical power can be achieved with 20 observations per cluster expected by theoretical arguments and considering a relatively large cluster separation. To this end, we assumed that objective and patient-centered 88 assessments can identify 3 phenotypes of joint torque: control without DM, DM2 with and without NDP. Since the influence of perimenopause and hormonal changes on strength in women is unclear [22], only men aged between 18 and 65 years were included in the study.

Eighty-eight participants were included in the study, with a mean age of 54.5 (9.28) years old, 29 of them were control subjects without DM2, 59 had DM2 (36 without DPN and 23 with DPN). They were also insufficiently active (should do at least 150 minutes of moderate-intensity or 75 minutes of vigorous-intensity aerobic exercise throughout the week) [23] and overweight (62.5%), with a mean body mass index (BMI) of 27.46 (3.71) kg/m^2 (Table 1).

Table 1. Description of cluster characteristics.

Variable	Global N=88	Cluster 1 N=29	Cluster 2 N=59	p
Age (years)	54.50 [44.75, 59.00]	48.00 [40.00, 58.00]	56.00 [50.00, 59.00]	0.06
DM2	54 (61.4)	0 (0.0)	54 (91.5)	< 0.001
Without DPN	65 (73.9)	29 (100.0)	36 (61.0)	< 0.001
With DPN	23 (26.1)	0 (0.0)	23 (39.0)	< 0.001
Mild	10 (11.4)	0 (0.0)	10 (16.9)	
Moderate	7 (8.0)	0 (0.0)	7 (11.9)	
Severe	6 (6.8)	0 (0.0)	6 (10.2)	
Fuzzy	0.67 [0.67, 2.16]	0.67 [0.67, 0.67]	0.78 [0.67, 4.47]	< 0.001
HbA1C (%)	6.45 [5.47, 8.33]	5.30 [5.10, 5.60]	7.70 [6.45, 9.45]	< 0.001
MNSI	1.00 [0.00, 4.00]	0.00 [0.00, 1.00]	3.00 [1.00, 5.00]	< 0.001
Physical activity				0.3
Sufficiently active	33 (37.5)	13 (44.8)	20 (33.9)	
Insufficiently active	55 (62.5)	16 (55.2)	39 (66.1)	
On medication				< 0.001
Yes	35 (39.8)	0 (0.0)	35 (59.3)	
No	53 (60.2)	29 (100.0)	24 (40.7)	
Anthropometric measurements				
Body mass (kg)	83.00 (12.40)	79.30 (12.00)	84.82 (12.29)	0.05
Height (m)	1.72 (0.08)	1.72 (0.07)	1.72 (0.08)	0.9
BMI (Kg/m^2)	27.46 [25.65, 29.65]	26.25 [24.98, 28.70]	27.86 [25.90, 30.10]	0.01
Classification				0.03
Not obese				
Normal	11 (12.5)	8 (27.6)	3 (5.1)	
Overweight	55 (62.5)	16 (55.2)	39 (66.1)	
Obese				
Grade I Obesity	18 (20.5)	4 (13.8)	14 (23.7)	
Grade II Obesity	3 (3.4)	1 (3.4)	2 (3.4)	
Grade III Obesity	1 (1.1)	0 (0.0)	1 (1.7)	
Peak torque (N.m)				
Isometric				
Extension				
Knee	16.41 [13.32, 267.82]	289.04 [259.67, 316.17]	13.71 [12.32, 16.41]	< 0.001
Ankle	15.09 [4.04, 123.44]	147.78 [122.21, 164.89]	8.10 [3.01, 15.09]	< 0.001
Flexion				
Knee	14.80 [12.23, 102.82]	116.30 [101.19, 135.42]	13.09 [11.67, 14.80]	< 0.001
Ankle	12.81 [3.23, 41.26]	44.31 [39.78, 55.00]	8.21 [2.93, 12.81]	< 0.001
Concentric				
Extension				
Knee	189.15 [156.73, 231.94]	230.46 [196.73, 261.18]	169.46 [144.01, 198.55]	< 0.001
Ankle	72.64 [52.85, 91.23]	94.86 [80.34, 109.63]	58.78 [44.90, 74.70]	< 0.001
Flexion				
Knee	97.89 [74.67, 116.18]	116.84 [98.56, 135.85]	91.28 [70.48, 104.06]	< 0.001
Ankle	20.91 [17.03, 26.66]	19.45 [13.42, 23.91]	22.39 [19.04, 27.13]	0.02
Cytokines				
TNF-α	0.74 [0.71, 0.74]	0.71 [0.71, 0.71]	0.74 [0.74, 0.87]	< 0.001
IL-1β	0.87 [0.87, 0.90]	0.87 [0.87, 0.87]	0.90 [0.87, 0.90]	0.002
IL-6	0.73 [0.51, 0.73]	0.51 [0.51, 0.51]	0.73 [0.73, 0.74]	< 0.001

Continuous variables are presented as median [interquartile range]; categorical variables as counts (percentage).

Exclusion criteria

Exclusion criteria were self-reported chronic diseases with systemic repercussions (unstable angina, uncontrolled systemic hypertension and kidney failure), osteoarticular diseases (rheumatoid arthritis, arthrosis, history of fractures, herniated discs and orthopedic leg surgeries), vascular diseases, use of medications that alter muscle strength (antihypertensive β-blockers, testosterone replacement and use of GH and IGF-1 growth hormones). The absence of pre-diabetes, nondiabetic neuropathy and BMI >35 kg/cm² were tested during clinical assessment.

Clinical assessment protocol

The presence and absence of DM2 were confirmed by an endocrinologist, in accordance with the criteria of the American Diabetes Association (2014). After confirmation, participants were screened by a physiotherapist, which included anamnesis, a physical examination, and assessment for the presence of DPN.

DPN assessment

DPN was clinically evaluated using the Michigan Neuropathy Screening Instrument (MNSI), in addition to symptom assessment and a physical exam [24]. The physical exam involved evaluating tactile sensitivity via esthesiometry, using a 10 g monofilament in 4 plantar areas (surface of the hallux and 1st, 3rd and 5th metatarsal heads) [25] and vibratory sensitivity with 128Hz vibration in the medial region of the hallux interphalangeal joint (34). Given that DPN progresses slowly and homogeneously [26,27], it can be difficult to classify its severity based solely on the sum of scores [13,28]. In order to classify DPN severity, an artificial intelligence decision support system based on *fuzzy* logic was used [13,28]. The clinical variables (DPN symptoms and perceived tactile and vibratory sensitivity) obtained in the MNSI protocol were used as input variables for this system. Combined analysis of these data generates a pertinence score from 0 to 10 that establishes a clinical diagnosis of the DPN severity [28].

Proinflammatory cytokines

Plasma concentrations of TNF- α, IL-1β and IL-6 were analyzed by sandwich ELISA

(enzyme-linked immunosorbent assay) and each cytokine was tested according to the manufacturer's instructions [29]. Readings were performed using a 490 nm filter. The detection limits of plasma cytokines were: IL-1ß -5pg/ml; IL-6 -2.62 pg/ml; TNF – α -1.7 pg/ml. After analyses, the data were transformed and normalized by the standard curve.

Torque assessment

An isokinetic dynamometer (Biodex Multi-joint System III) was used for torque assessment. The equipment was calibrated according to the manufacturer's recommendations prior to assessment. Concentric torque at 60°/s and isometric torque in flexion and extension of the dominant knee and ankle were evaluated [15].

Knee torque was evaluated with subjects seated on the dynamometer chair, their trunk and hips stabilized by straps, the latter flexed at approximately 85° [15]. Knee flexion at 90º was considered position 0. The rotation axis of the isokinetic dynamometer was aligned with the lateral epicondyle of the femur and the lever arm firmly attached to the third distal of the leg (5 cm above the lateral malleolus).

For ankle torque assessment, participants were seated on the dynamometer with their trunk and hips stabilized by straps, the latter flexed at approximately 70° [15]. The knee was attached to the rubber support of the equipment and maintained at 30° flexion, and the foot to a platform in a neutral position considered position 0 (where the ankle forms a 90° between the leg and foot). The rotation axis of the isokinetic dynamometer was aligned with the lateral malleolus [15].

Isometric knee torque was assessed at 30° extension from position 0 and the ankle in a neutral position. The concentric torque was accessed at 70° range of motion (ROM) for the knee and 45° for the ankle. The testing order for the joint movements and type of muscle contraction was previously randomized for each subject. Torque data were processed in a MATLAB software routine (version7.0.1) to obtain the largest peak torque of 5 repetitions.

Statistical analysis

One of the objectives of this study was to investigate the association between torque and the presence of DM2. To that end, principal component analysis (PCA) was applied to assess the importance of each torque (isometric and concentric knee and ankle flexors and

extensors) in explaining most of the total variability of torque data. Before PCA, Bartlett's sphericity test and the Kaiser-Meyer-Olkin (KMO) measure of sampling adequacy were used to confirm whether PCA was suited to the data set. With respect the CPA, Jolliffe's (1992) recommendation was adopted [30], whereby the number of variables to be discarded should be equal to the number of components with variance (Eigenvalue) less than 0.7. The variables that exhibited the greatest correlation with the principal components with the smallest variances were discarded because their variation was practically insignificant [30].

Next, cluster analysis was performed using the variables identified in PCA as being responsible for most of the total variation. The ideal number of clusters (k) was determined by silhouette analysis and the internal stability of each cluster was tested using the Jaccard index. Once the number of clusters and centroids was established, patients were classified into groups according to proximity functions using k-means clustering.

Continuous variables are presented as mean (standard deviation) or median [interquartile range], in accordance with the Shapiro-Wilk normality test, and categorical variables as counts and percentages. Intercluster comparisons were performed by the Wilcoxon-Mann-Whitney test for continuous variables and Pearson's chi-squared with Yates' continuity correction for categorical variables.

Stepwise regression was used to investigate the factors associated with HbA1c levels (%) and MNSI scores. The covariables included in the final model were selected based on minimization of Akaike's Information Criteria (AIC). Statistical significance was set at $p < 0.05$. All analyses were conducted using R software version 3.5.3 (The R Foundation for Statistical Computing, Vienna, Austria) and R-studio 1.1.463 (RStudio Inc., Boston, USA).

Results

Principle Component Analysis

Bartlett's sphericity test ($p < 0.001$) and KMO (0.8) confirmed that the data set was suited to the technique (Table 2).

Table 2. Suitability and sphericity of the data set

Principal component	$Lambda_i$	% var. PC	% var. PC (cumulative)
PC1	4.74	59.24	59.24
PC2	1.45	18.10	77.34
PC3	0.90	11.25	88.59
PC4	0.38	4.72	93.31
PC5	0.30	3.70	97.02
PC6	0.12	1.56	98.58
PC7	0.09	1.11	99.69
PC8	0.02	0.31	100.00

Principal components (PC), Eigenvalues ($Lambda_i$) and percentage variance explained by the components (% var. PC) of torque variables.

Cluster identification and characterization

Two clusters were identified based on the behavior of peak concentric knee flexion and extension torque and peak isometric ankle extension torque: Cluster 1 (n= 29) and Cluster 2 (n= 59). Cluster 1 consisted only of control individuals and Cluster 2 included all DM2 subjects: 23 with and 36 without DPN. The clusters were similar in terms of age ($p= 0.06$) and physical activity level ($p= 0.3$), but Cluster 2 exhibited high body weight ($p= 0.05$), and BMI ($p\leq 0.01$), increased levels of HbA1C ($p\leq 0.01$) and cytokines TNF-α ($p\leq 0.01$), IL-1β ($p\leq 0.01$) and IL-6 ($p\leq 0.01$) (Table 1). In terms of mechanical parameters, Cluster 2 showed lower peak torques at concentric knee flexion ($p\leq 0.01$) and extension ($p\leq 0.01$) and isometric ankle extension ($p\leq 0.01$) (Table 1).

Factors associated with HbA1c levels

The relationship between HbA1c levels and the remaining variables was assessed based on the previous results obtained, with 46% (adjusted $R^2 = 0.43$) of variation explained by age, body mass, increased plasma IL-6 concentrations, peak concentric knee flexion and extension torque and peak isometric ankle extension torque (Table 3).

Table 3. Final stepwise regression model.

Dependent variable	**R^2**	**Adjusted R^2**	**p**	
HbA1c (%)	0.46	0.43	< 0.001	
Independent variables	**β Coefficient**	**Standard error**	**CI 95%**	**p**
Age (years)	-0.06	0.02	-0.11 - -0.01	0.01
Body mass (kg)	-0.05	0.02	-0.09 - -0.02	0.004
Peak concentric knee extension torque	-0.01	0.00	-0.02 - 0.00	0.04
Peak isometric ankle extension torque	-0.02	0.01	-0.03 - -0.01	0.001
IL-6	+4.33	2.80	-1.23 - 9.90	0.13

Factors associated with the MNSI score

The relationship between MNSI score and the remaining variables was also analyzed, with 54% (adjusted $R^2 = 0.51$) of variation explained by reduced peak concentric knee extension torque and peak isometric ankle extension as well as increased plasma TNF-α and IL-6 levels (Table 4).

Factors associated with HbA1c levels

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Table 4. Final stepwise regression model.

Dependent variable	R^2	Adjusted R^2	p	
MNSI score	0.54	0.51	< 0.001	
Independent variables	**ß Coefficient**	**Standard error**	**CI 95%**	**p**
Concentric knee extension torque	-0.01	0.00	-0.02 - 0.00	0.07
Isometric ankle extension torque	-0.01	0.01	-0.02 - 0.00	0.03
TNF-α	+7.94	2.65	2.67 - 13.21	0.003
IL-6	+4.81	3.28	-1.71 - 11.32	0.15

MNSI= Michigan Neuropathy Screening Instrument

Discussion

The main results of this study demonstrated that concentric knee flexion and extension torques and isometric ankle extension torque distinguished between individuals with and without DM2 with an accuracy of 94.3% (95% IC 89.5% - 99.2%), but could not distinguish between DM2 subjects with and without DPN. Additionally, HbA1C level was associated with a decline in these torques, increased age and high plasma IL-6 concentrations. DPN symptoms were associated with a rise in the inflammatory markers IL-6 and TNF-α and decreased concentric and isometric knee and ankle extension torque, respectively. Contrary to previous studies [1–3], these original findings confirmed that an impaired joint torque is associated with a set of factors, most importantly to DPN and subclinical inflammation, but not to glycemic control independently.

We confirmed our hypothesis that torque values would differentiate between individuals with and without DM2 and that HbA1C and the presence of DPN would be associated with lower torques and increased plasma proinflammatory cytokine levels, since studies have shown lower torque in these individuals when compared to controls without DM2 [14–16]. Although the DM2 sample contained individuals with and without DPN, torque could not differentiate between these conditions, demonstrating that reduced torque is more strongly correlated with DM2 than DPN. Recent studies have shown that muscle

endurance [31] and the complexity of strength and electromyographic activation [32] are reduced in DM2 individuals, regardless of the presence of DPN and only the muscle power reduction seems to be more related to the severity of DPN [31]

Although torque was lower in subjects with DM2, only concentric knee flexion and extension and isometric ankle extension were able to differentiate between individuals in terms of DM2 presence. Muscle strength is heavily influenced by an individual's physical behavior [7] and concentric knee flexion and extension and isometric ankle extension are important to gait and lower limb function [19]. Meta-analyses [8,9] indicated that low intensity activities involving the muscles that participate in these movements, such as the soleus, vastus intermedius and rectus femoris muscle [10], are essential to controlling metabolic syndrome and preventing DM2, due to the high number of oxidative fibers [10].

Although another study also reported a strong association between concentric knee extension torque [3] and HbA1C level, suggesting that extension torque can be used to screen for DM2, our study is the first to test this hypothesis using different movements and muscle contractions, such as concentric and isometric knee and ankle flexion and extension.

The association between reduced concentric knee extension and isometric ankle extension torques and increased HbA1C reinforces that torque in both movements and contraction types can be used for screening purposes. Other studies have reported an association between reduced strength and increased HbA1C levels [1–3] in individuals with DM2, but ours is the first to show that reduced torque is associated with a set of factors, including DPN symptoms and increased subclinical inflammation, and not glycemic control alone. Although the effect of DPN on torque reduction is controversial, reduced torque was associated with DPN [33] and recent studies demonstrate that decreased torque in DM2 individuals was independent of DPN [14–16]. Although this result indicates that DPN also leads to a decline in torque, the influence of DPN was not sufficiently significant to generate a third cluster differentiating between DM2 individuals with and without DPN.

According to the American Diabetes Association (ADA) (2014), HbA1C is the most accurate blood test to detect DM2 and values from 5.6 - 5.9% indicate increased risk of developing the disease, where preventive interventions can still be effective [34]. Although HbA1C level is a well-established indicator, hyperglycemia means it develops slowly without the patient exhibiting classic symptoms (ADA, 2014). The present study shows that

torque has a similar capacity to detect DM2 as HbA1C levels, suggesting that this variable can be used to support early DM2 diagnosis or risk detection. Isometric ankle extension torque is easy to assess with portable dynamometers and useful in epidemiological studies and screening campaigns for the disease.

A limitation of the present study is its cross-sectional design, which does not provide evidence of a temporal relationship between torque and DM2. However, bias due to variations between independent and dependent variables in a same individual was reduced. Additionally, although the isokinetic dynamometer is considered the best tool for assessing torque, its high cost means it can only be used in laboratories or specialized centers. As such, the device was used to analyze isometric torque because this measurement can be tested in clinical settings, companies and basic health units using simpler and less costly tools such as portable dynamometers. Further studies that analyze this measurement in different settings, with individuals of both sexes, varying age ranges and using portable dynamometers will provide an important contribution to this topic.

Conclusion

Concentric knee flexion and extension and isometric ankle extension torques can distinguish between subjects with and without DM2, but not with and without DPN. Reduced torques in individuals with DM2 associated with poor glycemic control, subclinical inflammation and DPN symptoms were not sufficiently significant to distinguish between DM2 individuals with and without DPN. Concentric and isometric knee and ankle torque evaluation can be associated with blood tests to support the early DM2 screening.

Data Availability

The datasets used to support the findings of this study are available from the corresponding author upon request.

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Authors' contributions

J. P. F, H.P.J. and T.F.S are the guarantors of this study and, as such, had full access to all the data and assumes responsibility for its integrity, accuracy and analysis. J.P.F., T.F.S., H.P.J, P.R.M.S. and I.C.N.S. were responsible for the study design, data analysis and interpretation and the literature review. A.M.O.L. and J.P.F. were responsible for data collection and the reliability of measurements, H. P. J. and R. A. S. F. were responsible for data processing statistical analyses. S.R. and I.C.N.S were responsible for the final review. All the authors contributed to the manuscript.

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CONCLUSÃO

Indivíduos com DM2 e NDP apresentam maior rigidez e torque passivo nos movimentos do joelho e tornozelo em relação a controles sem DM e DM2 sem NDP. Os comprometimentos mecânicos das estruturas passivas foram mais evidentes em velocidades lentas, como a 5°/s. Os torques concêntrico e isométrico de flexão e extensão do joelho e isométrico de extensão do tornozelo podem distinguir indivíduos com e sem DM2, mas não DM2 com sem a NDP. A redução do torque em indivíduos DM2 está associado com pobre controle glicêmico, aumento da inflamação subclínica e sintomas da NDP. Embora o torque reduzido esteja associado com os sintomas da NDP, as alterações do torque não foram significantes ao ponto de distinguir DM2 com e sem NDP.

CONSIDERAÇÕES FINAIS

Esta análise do comportamento do torque passivo do joelho e tornozelo em indivíduos DM2 com e sem NDP permitirá compreender a manutenção do torque excêntrico, previamente observada nesta população e também que estudos de modelagem biomecanica sejam mais realisticos quanto as propriedades mecânicas do tecido musculoesquelético distal nesta população. Além disso, este resultado indica que investigações sobre o torque passivo e a rigidez articular em indivíduos com DM2 podem complementar as avaliações mais comuns de ativação muscular, reforçando a necessidade de medidas preventivas para se retardar ou prevenir os comprometimentos musculoesqueléticos relacionados ao aumento da rigidez durante a progressão da DM e NDP.

Nosso estudo mostra que é possível distinguir indivíduos com e sem DM2 por meio de medidas dos torques concentrico e isométrico do joelho e tornozelo. Seria muito interessante associar a avaliação da função concêntrica e isométrica aos exames sanguíneos, com a finalidade de apoiar o rastreio e diagnóstico precoce da DM2. Considerando que o comportamento físico pode afetar diretamente o controle glicêmico e a função muscular. Nossos resultados demonstram que a função muscular está diretamente ligada com a DM2,

futuros estudos analisando indivíduos de ambos os sexos e se ações voltadas para a redução do comportamento sedentário tendo e também tendo como objetivo manter ou melhorar a função muscular possa contribuir para a redução da incidência de DM2. No entanto, este é um tema que necessita.

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ELEMENTOS PÓS-TEXTUAIS

ANEXOS

Manuscrito 3 – Artigo Publicado no Brazilian Journal of Medical and Biological Research

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Decreased muscle strength is associated with proinflammatory cytokines but not testosterone levels in men with diabetes

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Abstract

The aim of this study was to compare muscle strength in male subjects with type 2 diabetes mellitus (DM2) with and without low plasma testosterone levels and assess the relationship between muscle strength, testosterone levels, and proinflammatory cytokines. Males (75) aged between 18 and 65 years were divided into 3 groups: control group that did not have diabetes and had a normal testosterone plasma level (>250 ng/dL), DnormalTT group that had DM2 with normal testosterone levels, and the DlowTT group that had DM2 and low plasma testosterone levels (<250 ng/dL). The age (means $\pm$ SD) of the groups was 48.4 ± 10, 52.6 ± 7, and 54.6 ± 7 years, respectively. Isokinetic concentric and isometric torque of knee flexors and extensors were analyzed by an isokinetic dynamometer. Plasma testosterone and proinflammatory cytokine levels were determined by chemiluminescence and ELISA, respectively. Glycemic control was analyzed by glycated hemoglobin (HbA1C). In general, concentric and isometric torques were lower and tumor necrosis factor (TNF)-α, interleukin (IL)-6, and IL-1β plasma levels were higher in the groups with diabetes than in controls. There was no correlation between testosterone level and knee torques or proinflammatory cytokines. Concentric and isometric knee flexion and extension torque were negatively correlated with TNF-α, IL-6, and HbA1C. IL-6 and TNF-α were positively correlated with HbA1C. The results of this study demonstrated that muscle strength was not associated with testosterone levels in men with DM2. Low muscle strength was associated with inflammatory markers and poor glycemic control.

Key words: Diabetes; Testosterone; Muscle strength; Cytokines

Introduction

Clinical and epidemiological evidence demonstrates that men with type 2 diabetes mellitus (DM2), metabolic syndrome, and obesity exhibit low plasma testosterone levels (1). Low testosterone levels are associated with metabolic and cardiovascular complications, sexual dysfunction, risk of bone fracture, and reduced muscle strength (2).

Around 20% of people with DM2 show a decline in testosterone levels (3) from disease onset (2). The causal interactions between obesity, metabolic syndrome, DM2, and testosterone deficiency are complex. In short, increased activity of the aromatase enzyme in adipose tissue raises estradiol levels, which inhibits the hypothalamic-pituitary-adrenal axis and prompts a decline in testicular production of testosterone. Additionally, hormones (leptin) and inflammatory mediators such as interleukin (IL)-1, IL-6, and tumor necrosis factor (TNF)-α in adipose tissue can also compromise testicular function (4).

Testosterone replacement therapy (TRT) can improve libido, sexual function, bone density, muscle mass preservation, body composition, mood, erythropoiesis, cognition, and quality of life as well as can lower the risk of cardiovascular disease (5) in obese men with DM2 (5,6). However, the topic is controversial because TRT is associated with increased risk of prostate cancer by worsening symptoms of benign prostatic hypertrophy, liver toxicity, hyperviscosity, erythrocytosis, severe heart failure, and cardiovascular disease, and exacerbates untreated sleep apnea, as previously reviewed (7–9). Thus, physicians should

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discuss symptom severity with the patients, including those resulting from loss of muscle strength, and weigh the potential risks and benefits of TRT (5,6).

Regardless of other factors, people with low testosterone levels develop reduced muscle strength (10). This is because testosterone stimulates protein synthesis and the recruitment of satellite cells (11). Additionally, testosterone inhibits production of the inflammatory cytokines IL-1 and IL-6 (12), which are known to activate apoptosis pathways and muscle atrophy (13). However, there is no study that analyzed the muscle strength of people with DM2 and the disease relationship to plasma testosterone levels. Although some research has shown that individuals with DM2 exhibit reduced muscle strength (14), whether low testosterone levels can exacerbate loss of strength in these subjects has yet to be established. Isokinetic dynamometry is a safe, reliable, and reproducible method to assess joint torque, providing the strength of individuals under different types of contraction (15). In addition, knee muscle strength plays an important role in movement and quality of life.

Therefore, this study aimed to evaluate the concentric and isometric torque of knee flexion and extension in diabetic men with and without hypogonadism. The presence of subclinical inflammation in DM2 and its influence on the musculoskeletal system is well known, activating pathways of apoptosis muscle atrophy (13) and reducing testicular testosterone secretion (16). As such, the present investigation analyzed participants' plasma levels of the proinflammatory cytokines IL-1, IL-6, and TNF-α.

Given that its association with subclinical inflammation means that DM2 can affect muscle strength (17), and that hypogonadism can also affect muscle strength (18) independently of DM2, the hypothesis of the study was that knee flexion and extension strength is lower in subjects with both DM2 and hypogonadism compared to subjects with DM2 and control subjects without hypogonadism.

Material and Methods

Subjects

The participants were recruited from local healthcare units and the Endocrinology Clinic of the Federal University of São Carlos. Males aged between 18 and 65 years were included in the study. The inclusion criteria for the control group were men without DM2 (19) and with normal total testosterone levels (20), for the DnormalTT group, men with DM2 (19) and normal total testosterone levels, and for the DlowTT group, men with DM2 (19) and low total testosterone levels (20). A total of 287 subjects were interviewed for eligibility. The exclusion criteria were cardiac diseases, pulmonary emphysema, knee arthrosis or arthritis, a history of knee ligament or meniscus injuries, herniated discs, stroke, peripheral diabetic neuropathy (PDN), and anti-inflammatory or hormonal therapy. The 75 subjects who met the inclusion criteria were distributed into the three groups: Control (n=20), DnormalTT (n=45), and DlowTT (n=10) (Figure 1).

The study complied with the Guidelines and Regulations for Research Involving Human Subjects (National Health Council Resolution 196/1996) and was approved by the university's Research Ethics committee (protocol No. 797.125). All participants took part voluntarily and gave written informed consent.

Clinical evaluation

PDN was evaluated by a trained physical therapist using the following clinical parameters: i) typical neuropathy symptoms assessed by a questionnaire based on the Michigan Neuropathy Screening Instrument (21); ii) tactile sensitivity using a 10 g Semmes-Weinstein monofilament (Sorri-Baru, Brazil) tested in four plantar areas (hallux plantar face, and the 1st, 3rd, and 5th metatarsals); and iii) vibratory perception with a 128 Hz tuning fork applied to the medial region of the hallux interphalangeal joint (21). To determine the presence of PDN, these variables were processed in artificial intelligence Fuzzy Logic System software (LaBiMPH, Brazil), described in greater details in previous studies (22,23). The software combines each fuzzy set of the input variables and gives the degree of PDN between 0 and 10, classified as follows: i) <2.5 absent; ii) 2.5–5.0 mild; iii) 5.1-8.0 moderate; iv) >8.0 severe (22). The degree obtained by the fuzzy model showed a very strong correlation with the expert's assessment (Pearson's coefficient r=0.943) and a high level of accuracy when classifying real patients analyzed with the model (ROC curve area=0.91) (22).

Anthropometric and glycemic control data

Body mass index (BMI) was calculated as follows: weight (kg) / height^2 (cm). The glycated hemoglobin (HbA1c) percentage is a 3-month indicator of glucose control and was measured in all participants (19).

Plasma testosterone

Total plasma testosterone level was determined by chemiluminescence (24). Data are reported in ng/dL and total testosterone <250 ng/dL was considered low (20).

Proinflammatory cytokines

Plasma concentrations of TNF-α, IL-1β, and IL-6 were analyzed by sandwich ELISA (enzyme-linked immunosorbent assay) and each cytokine was tested according to the manufacturer's instructions (25). Readings were performed using a 490-nm filter. The detection limits of the cytokines in the serum were 5 pg/mL for IL-1β, 2.62 pg/mL for IL-6, and 1.7 pg/mL for TNF-α. After analyses, the data were transformed and normalized by the standard curve.

Torque analysis

Concentric and isometric torque of knee flexors and extensors were analyzed using an isokinetic dynamometer

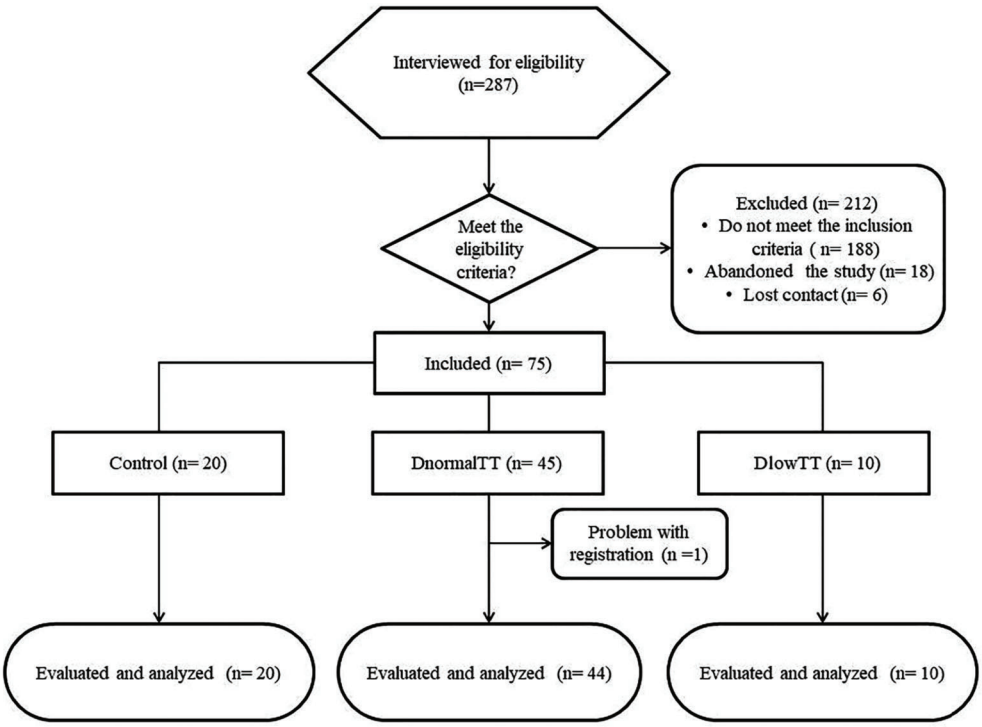

Figure 1. Flowchart of the study design. Control: control group; DnormalTT: diabetics with normal total testosterone; DlowTT: diabetics with low total testosterone.

(Biodex, System III, USA). Concentric torque was analyzed at 60°/s. The equipment was calibrated according to manufacturer's recommendations before the assessments. Individuals were asked which leg they could kick a ball hardest in order to determine their dominant limb. The type of contraction tested first was chosen randomly using a randomization spreadsheet. Before maximal testing, submaximal familiarization sessions were conducted with 3 repetitions for each movement in the concentric mode and 2 repetitions in the isometric mode. A 1.5-min rest was allowed between modes and 3 min between familiarization and the maximal test. During the test, participants remained seated in the dynamometer chair with the backrest at 85°, trunk stabilized and fixed to the backrest using two belts with an additional pelvic belt. The axis of rotation of the dynamometer was aligned with the lateral epicondyle of the femur and the resistance arm fixed to the distal third of the leg, just above the malleolus.

Verbal and visual encouragement was provided during all maximal voluntary contractions, always by the same evaluator. After the analyses, the text files of the tests were processed in MATLAB (version 7.0.1, MathWorks, USA) to determine the peak torque achieved by participants in the repetitions. Peak torque in N-m (newton meters) was normalized by the weight of the individual and multiplied by 100.

Statistical analysis

The Levene and the Kolmogorov-Smirnov tests were applied to determine homogeneity of variance and normal distribution, respectively. One-way ANOVA and Tukey's post-hoc test were performed to compare the clinical and demographic characteristics of the groups, as well as concentric and isometric torque of the knee flexors and extensors. Significance was set at 5%. Effect sizes (Hedges' g) of torque values between groups were also calculated, and considered insignificant (0.00–0.19), small (0.20–0.39), medium (0.40–0.79), or large ($\geqslant$ 0.80) (26).

Kruskal-Wallis and Mann-Whitney U tests were applied to analyze the proinflammatory cytokines. For nonparametric comparisons, the significance level was adjusted according to the number of comparisons and set at $P \leqslant 0.016$. Correlations between concentric and isometric knee flexion and extension torque, proinflammatory cytokines, and HbA1c were assessed using Pearson's correlation coefficient (r=0.10–0.29: low correlation; r=0.30–0.49: medium correlation; r=0.50 –1: high correlation) (27).

Results

One individual in the DnormalTT group was unable to complete the test. All groups were similar in terms of age. The DlowTT group had a higher BMI than the control

Table 1. Clinical characteristics of the participants.

	Control (n=20)	DnormalTT (n=44)	DlowTT (n=10)	ANOVA
Age (years)	48.40 (10.03)	52.61 (7.81)	54.60 (7.24)	F=2.39; P=0.09
Time since diagnosis (months)	0.0 (0)	108.90 (72.48)*	89.40 (75.17)*	F=21.15; P=0.00
Testosterone (ng/dL)	402.0 (292.7)	369.2 (84.2)	204.6 (44.2)*#	F=0.60; P=0.55
HbA1C (%)	5.3 (0.4)	8.5 (2.5)*	9.1 (2.2)*	F=2.74; P=0.07
BMI (kg/m^2)	26.4 (4.1)	27.8 (3.1)	30.7 (5.0)*	F=4.42; P=0.01
Degree of peripheral neuropathy (Fuzzy score)	0.67 (0.2)	1.2 (0.9)*	0.8 (0.2)	F=4.39; P=0.01
Oral antidiabetic / insulin / oral (n)		39/0/5	9/0/1	

Data are reported as means ± SD. DnormalTT: type 2 diabetics with normal total testosterone; DlowTT: type 2 diabetics with low total testosterone. *$P<0.05$ compared to controls; #$P<0.05$ compared to DnormalTT.

Table 2. Peak torque for the different contraction types.

Type of Contraction	Joint movement	Control (n=20)	Effect size Control *vs* DnormalTT	DnormalTT (n=44)	Effect size DnormalTT *vs* DlowTT	DlowTT (n=10)	Effect size Control *vs* DlowTT	ANOVA
Concentric	Flexion	94.18 (33.87)	–1.58	53.35 (21.16)*	0.59	66.67 (28.37)*	0.85	F=16.8; P=0.00
	Extension	156.61 (56.50)	–1.17	104.36 (37.68)*	0.74	132.98 (41.79)	0.45	F=10.0; P=0.00
Isometric	Flexion	99.65 (35.77)	–0.65	80.46 (25.99)	–0.33	70.81 (40.73)*	0.77	F=3.73; P=0.02
	Extension	218.90 (54.04)	–0.81	171.95 (58.88)*	0.17	181.65 (43.10)	0.73	F=4.89; P=0.01

Peak torque is reported as means ± SD (N.m/kg × 100). DnormalTT: type 2 diabetics with normal total testosterone; DlowTT: type 2 diabetics with low total testosterone. Effect size: insignificant (0.00–0.19), small (0.20–039), medium (0.40–0.79), large ($\geq$0.80). *$P<0.05$ compared to controls.

Table 3. Comparison of inflammatory markers tumor necrosis factor (TNF-α), interleukin- 6 (IL-6), interleukin 1- beta (IL-1β).

	Control (n=20)	DnormalTT (n=44)	DlowTT (n=10)	Kruskal-Wallis test
TNFα (pg/mL)	0.71 (0.71–0.71)	0.74 (0.71–1.00)*	1.00 (0.74–1.00)*	H=46.2; $P<0.01$
IL-6 (pg/mL)	0.50 (0.51–0.51)	0.73 (0.51–0.83)*	0.73 (0.73–0.83)*	H=45.0; $P<0.01$
IL-1β (pg/mL)	0.87 (0.87–0.87)	0.90 (0.84–0.90)*	0.90 (0.84–0.90)*	H=14.0; $P<0.01$

Data are reported as means (minimum–maximum). DnormalTT: type 2 diabetics with normal total testosterone; DlowTT: type 2 diabetics with low total testosterone. *$P<0.016$ compared to controls.

group ($P\leq0.01$); however, there was no difference in BMI between the two groups with DM2. Intergroup differences were observed for HbA1C values, but only between the groups with DM2 and the control group ($P<0.01$; Table 1).

Peak torque was similar for all movements and both contraction types in the groups with DM2. When compared to controls, the DnormalTT group showed lower concentric and isometric knee extension torques ($P<0.01$) and the DlowTT exhibited lower isometric knee flexion torque (P=0.04). Both groups with DM2 showed lower concentric knee flexion torque than controls ($P<0.02$; Table 2).

TNF-α, IL-6, and IL-1β concentrations were higher in the groups with DM2 than controls ($P<0.01$), but no difference was found between the DnormalTT and DlowTT groups (Table 3). HbA1c was positively correlated with IL-6 and TNF-α (r=0.55, $P<0.01$; r=0.59, $P<0.01$) and negatively correlated with concentric knee flexion and extension torque (r=–0.30, $P<0.01$; r=–0.24, P=0.03). Concentric and isometric knee flexion and extension torque were negatively correlated with TNF-α (r=–0.27, $P<0.01$; r=–0.33, $P<0.01$; r=–0.35, $P<0.01$; r=–0.43, $P<0.01$, respectively, for TNF-α *vs* concentric knee flexion; TNF-α *vs* concentric knee extension; TNF-α *vs* isometric knee flexion, and TNF-α *vs* isometric knee extension) and IL-6 (r=–0.22, P=0.03; r=–0.27, $P<0.01$; r=–0.38, $P<0.01$; r=–0.41, $P<0.01$, respectively, for IL-6 *vs* concentric knee flexion; IL-6 *vs* concentric knee extension; IL-6 *vs* isometric knee flexion, and IL-6 *vs* isometric knee extension).

There was no correlation between testosterone level and knee torques, or proinflammatory cytokines, considering all groups.

Discussion

The results of the present study showed reduced isometric and concentric torque in individuals with DM2 regardless of testosterone levels and their association with high IL-6 and TNF-α concentrations. These findings were consistent with the important pathophysiological role of inflammation in reducing muscle strength.

With respect to decreased muscle strength in individuals with DM2, our results confirmed the findings of previous studies showing that individuals with diabetes have lower skeletal muscle strength than those without diabetes (28,29). The mechanism of muscle strength decline in DM2 subjects is not well defined. In the present study, clinical neuropathy was excluded, but subclinical neuropathy could not be excluded.

We hypothesized that low testosterone levels would negatively influence decreased muscle strength in individuals with DM2. However, both diabetic groups (normal and low testosterone subgroup) exhibited lower muscle strength than control subjects and there was no correlation between testosterone levels and muscle strength. Testosterone administration has been associated with increased muscle strength. However, previous studies (10,30) that examined the correlation between endogenous testosterone and muscle strength have been inconclusive, possibly because circulating testosterone levels may not directly or linearly reflect its biological action on target tissues.

Mechanistically, the interaction between low muscle strength and high HbA1c may be explained by the effect of hyperglycemia on skeletal muscle mitochondrial dysfunction, protein degradation, and autophagy pathways (31,32), as well as the accumulation of advanced glycation products and oxidative stress (33).

Another potential mechanism for decreased muscle strength in individuals with DM2 is the rise in inflammatory cytokine levels. In the present study, the inflammatory markers TNF-α, IL-6, and IL-1β concentrations were higher in both groups with DM2 than in controls. It has been previously demonstrated that increased plasma concentration of inflammatory cytokines may cause loss of strength in these individuals (13,34). Inflammatory cytokines may lead to the inhibition of skeletal muscle protein synthesis and myoblast differentiation by activating MAFbx/atrogin-1, eIF3-f, MyoD, and MuRf1 (35–37). In addition, it has been reported that increased TNF-α levels may activate the caspase pathway, leading to skeletal muscle atrophy due to enzymatic fragmentation of muscle DNA (38,33).

In conclusion, the results of this study demonstrated that muscle strength was lower in individuals with DM2 and was not associated with low total testosterone levels. Our results also indicated that reduced muscle strength in subjects with DM2 subjects could be associated with an increase in the inflammatory cytokines IL-6 and TNF-α and poor glycemic control. However, further studies are needed to clarify this association.

As limitations, the DlowTT group was a very small sample and despite the number of individuals interviewed, only 10 individuals with DM2 and low testosterone met the eligibility criteria of the study. Given the sample size evaluated in the DlowTT group, one-way ANOVA as the statistical design, and an alpha error of 5%, the statistical power ($1 - \beta$) obtained using a small effect size was 0.70. We also calculated the sample size using a power of 0.93 and medium effect size (0.45). For future studies, we recommend using a sample size of 75 individuals equally distributed among groups. In addition, it would be interesting for future studies to analyze the association between strength and low testosterone level with the quality of life and physical activity level of these individuals.

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