PONTIFÍCIA UNIVERSIDADE CATÓLICA DO PARANÁ

ESCOLA DE SAÚDE E BIOCIÊNCIAS
PROGRAMA DE PÓS-GRADUAÇÃO EM ODONTOLOGIA
ÁREA DE CONCENTRAÇÃO EM BIOCIÊNCIAS

DÁFINE ZIELINSKI

# IMUNOEXPRESSÃO DA METALOPROTEINASE-8 EM LESÃO MUSCULAR DE RATOS SUBMETIDOS À NATAÇÃO

Curitiba
2014

**DÁFINE ZIELINSKI**

# IMUNOEXPRESSÃO DA METALOPROTEINASE-8 EM LESÃO MUSCULAR DE RATOS SUBMETIDOS À NATAÇÃO

**Dissertação apresentada ao Programa de Pós-Graduação em Odontologia, Área de concentração: Biociências, da Escola de Saúde e Biociências, da Pontifícia Universidade Católica do Paraná, como requisito parcial à obtenção do título de mestre em Odontologia.**

**Orientadora: Profa. Dra. Aline Cristina Batista Rodrigues Johann**

**Curitiba**

**2014**

Dados da Catalogação na Publicação
Pontifícia Universidade Católica do Paraná
Sistema Integrado de Bibliotecas – SIBI/PUCPR
Biblioteca Central

Z66i
2014

Zielinski, Dáfine
Imunoexpressão da metaloproteinase-8 em lesão muscular de ratos submetidos à natação / Dáfine Zielinski ; orientadora, Aline Cristina Batista Rodrigues Johann. – 2014.
56 f. : il. ; 30 cm

Dissertação (mestrado) – Pontifícia Universidade Católica do Paraná, Curitiba, 2014
Inclui bibliografias
Texto em português e inglês

1. Odontologia. 2. Úlceras. 3. Rato como animal de laboratório. 4. Natação. I. Johann, Aline Cristina Batista Rodrigues. II. Pontifícia Universidade Católica do Paraná. Programa de Pós-Graduação em Odontologia. III. Título.

CDD 20. ed. – 617.6

Pontifícia Universidade Católica do Paraná
Escola de Saúde e Biociências
Programa de Pós-Graduação em Odontologia

PUCPR
GRUPO MARISTA

**ATA DA SESSÃO PÚBLICA DE DEFESA DE DISSERTAÇÃO DE MESTRADO DO PROGRAMA DE PÓS-GRADUAÇÃO EM ODONTOLOGIA, DA PONTIFÍCIA UNIVERSIDADE CATÓLICA DO PARANÁ**

Exame de Dissertação n.º **206**

Aos quinze dias do mês de dezembro de 2014, realizou-se a sessão pública de defesa de dissertação "**IMUNOEXPRESSÃO DA METALOPROTEINASE-8 EM LESÃO MUSCULAR DE RATOS SUBMETIDOS À NATAÇÃO**", apresentada por **Dáfine Zielinski**, ano de ingresso **2013,** para obtenção do título de **Mestre em Odontologia** na Área de Concentração em **Biociências**. De acordo com as normas regimentais, a Banca Examinadora atribuiu e deliberou os seguintes conceitos:

Membros da Banca Examinadora:

| | | |
|---|---|---|
| Profª Drª Aline Cristina Batista Rodrigues Johann (PUCPR) **Orientador (a)** | APROVADA<br>Conceito | Assinatura |
| Profª Drª Ana Maria Trindade Grégio (PUCPR) | Aprovado<br>Conceito | Assinatura |
| Prof. Dr. João Armando Brancher (UP) | Aprovado<br>Conceito | Assinatura |

**Conceito Final**: Aprovado

Observações da Banca Examinadora:

A CANDIDATA DEVERÁ REALIZAR AS ALTERAÇÕES SUGERIDAS PELA BANCA NO PERÍODO DE 30 DIAS, ASSIM COMO ENTREGAR TODA A DOCUMENTAÇÃO NECESSÁRIA PARA RECEBER O TÍTULO DE MESTRE.

Profª Drª Aline Cristina Batista Rodrigues Johann
**Presidente da Banca Examinadora**

Prof. Dr. Sérgio Vieira

**SUMÁRIO**

# ARTIGO EM PORTUGUÊS

## TÍTULO: IMUNOEXPRESSÃO DA METALOPROTEINASE-8 EM LESÃO MUSCULAR DE RATOS SUBMETIDOS À NATAÇÃO


**AUTORES:**

1. Dáfine Zielinski
2. Aline Cristina Batista Rodrigues JOHANN, DDS, MSc, PhD[1]*

**AFILIAÇÃO INSTITUCIONAL**

[1]Pontifícia Universidade Católica do Paraná, School of Health and Biosciences, Rua Imaculada Conceição, 1155, Prado Velho 80.215-901, Curitiba, PR, Brazil.

**Endereço para correspondência**
Profa. Aline Cristina Batista Rodrigues Johann. PhD
Clínica de Odontologia
Pontifícia Universidade Católica do Paraná
Rua Imaculada Conceição, 1155, Prado Velho
80.215-901
Curitiba, PR, Brazil.
Telephone number: +55 41 3271-2592 Fax number: +55 41 3271-1405
E-mail: alinecristinabatista@yahoo.com.br

**Resumo**

A metaloproteinase 8 (MMP-8) é a colagenase predominante no processo de reparo de feridas, a qual degrada o colágeno tipo I, principal tipo de colágeno no tecido da ferida. Considerando que a atividade física interfere nesse processo, o presente estudo teve por objetivo verificar a imunoexpressão da MMP-8 nesse processo junto à essa atividade. Quarenta e oito ratos foram divididos em: a) AF – animais com lesão muscular e que havia praticado atividade física (natação) com carga progressiva; b) C – controle com animais sedentários com lesão muscular. Úlceras foram induzidas quimicamente (NaOH 40%) no gastrocnêmio. Nos dias 7, 14 e 21 após a indução da lesão muscular, os animais de ambos os grupos foram mortos. A área lesionada foi removida, processada histologicamente e submetida à imunoistoquímica usando anti-MMP8. Foram capturadas cinco imagens dos cortes, sendo avaliadas por um software analisador de imagens. O grupo AF (4,21± 1,46 $\mu m^2$) apresentou uma tendência de aumento da expressão de MMP-8 aos 14 dias comparado com o C (1,06± 1,32 $\mu m^2$; p=0,0565), e o oposto foi observado aos 21 dias, sendo 3,15± 0,62 $\mu m^2$ para AF e 5,63± 0,63 $\mu m^2$ para C (p=0,0021). Os resultados sugerem que a atividade física aumenta a expressão da MMP-8 aos 14 dias e diminui aos 21dias, o que pode ter acarretado em uma menor deposição de colágeno tipo I aos 21 dias observada em estudo prévio, o que confere menor resistência da ferida.

***Palavras-chave*:** úlcera, metaloproteinases, exercício, reparo.

## Introdução

As metaloproteinases (MMPs) compõem uma família de aproximadamente 24 enzimas (endopeptidases) classificadas em cinco grupos: colagenases [1, 8 e 13], gelatinases [2 e 9], estromelisinas [3, 10, 11 e 19], as de tipo membrana [14, 15, 16 e 17], matrilisima [7 e 12] e outras. Diferentes entre si estruturalmente e em suas funções, são responsáveis pela degradação dos componentes da matriz extracelular e das membranas basais (5, 12).

A MMP-8, também chamada de colagenase de neutrófilo, é uma colagenase intersticial que cliva os colágenos tipos I, II e III em fragmentos. Apesar da nomenclatura, a MMP-8 não é apenas liberada por neutrófilos, mas também por outros tipos celulares: fibroblastos, monócitos, macrófagos, células epiteliais, células endoteliais e plasmócitos em resposta a estímulos inflamatórios (8). A MMP-8, a colagenase predominante no processo de reparo de feridas, degrada do colágeno tipo I, principal tipo de colágeno no tecido da ferida. A superexpressão dessa enzima está relacionada com a patogênese de feridas que não cicatrizam e com menor deposição de colágeno tipo I na ferida e menor resistência da cicatriz (4).

Namba et al. (2014) (13) verificaram em ratos com úlcera induzida, uma menor deposição de colágeno tipo I aos 14 e 21 dias, comparando animais submetidos a atividade física com sedentários. Os autores concluíram que a ferida apresentava aos 21 dias menor resistência no grupo treinado. Nessa mesma amostra, Gama & Grégio (2013) (6) verificaram que o grupo treinado demonstrou diminuição da expressão de MMP-1 aos 14 dias e um aumento aos 21 dias, concluindo que a atividade física pode alterar a expressão da MMP-1, retardando o processo de reparo de feridas aos 14 dias. Diante desses achados, surgiu a necessidade de se avaliar se a expressão da MMP-8, que é uma das enzimas responsáveis pela clivagem do colágeno tipo I, poderia ter influência nessa menor deposição do colágeno tipo I no grupo submetido á atividade física.

O objetivo do presente estudo foi avaliar a imunoexpressão da MMP-8 no processo de reparo de feridas de animais submetidos à atividade física e de animais sedentários.

## Material e Método

O presente estudo foi aprovado pelo Comitê de Ética em Uso de Animais da Pontifícia Universidade Católica do Paraná (CEUA/PUCPR), sob o protocolo número 297/07. A amostra foi composta em blocos previamente processados e arquivados no Laboratório de Patologia Experimental da PUCPR (13). Foram utilizados 48 ratos Wistar adultos machos e divididos em 2 grupos, sendo: a) AF – animais com lesão muscular e que havia praticado atividade física (natação) com carga progressiva; b) C – controle com animais sedentários com lesão muscular. Cada grupo de animais foi subdividido em três subgrupos de 7, 14 e 21 dias após a indução da lesão muscular.

Os animais do grupo C foram submetidos a um ambiente aquático, porém prontamente retirados a fim de mimetizar o grupo treinado. Os animais do grupo AF foram submetidos a um esquema de natação durante os cinco dias da semana e descansando dois dias de acordo com Gonçalves & Luciano (1999) (7). As sessões de natação começavam a partir de 8:00 da manhã e duravam 60 minutos. Os animais eram inseridos em um recipiente de amianto (100 cm x 70 cm x 60 cm) com coluna de água de 40 cm a 32°C.

A adaptação dos animais foi realizada num período de cinco dias no ambiente aquático, aumentando progressivamente o tempo de treinamento (10 minutos ao dia). A partir do sexto dia foi preso ao tórax do animal um elástico uma carga de 5% do peso total do animal. Os animais eram pesados diariamente a fim de ajustar esta carga. Após a indução da úlcera no dia 11, a atividade física foi continuada de tal forma: 7 dias, 60 minutos de natação, totalizando 18 dias de atividade física; 14 dias, 60 minutos de natação, totalizando 25 dias de atividade física; 21 dias, 60 minutos de natação, totalizando 32 dias de atividade física.

Depois dos animais serem anestesiados com Tiopental sódico (80mg/kg, Cristália, Itapira, Brasil), a úlcera foi induzida por meio da aplicação subcutânea de 0,08 ml de NaOH 40% no gastrocnêmico do animal, onde juntamente ao músculo sóleo e extensor longo dos dedos se forma o chamado tríceps sural e juntos, mantém uma manutenção da postura e realização de movimentos (17).

Ao término da última sessão de treino, os animais foram sacrificados por overdose de Tiopental sódico (150mg/kg).

Reação Imunoistoquímica

As reações imunoistoquímicas para a marcação da proteína MMP-8 foram realizadas no Laboratório de Patologia Experimental da PUCPR. A partir de blocos de parafina obtiveram-se cortes histológicos de 4 µm de espessura, que foram desparafinizados (xilol, Biotec, Curitiba, Brasil) e hidratados (álcool em soluções decrescentes, Biotec, Curitiba, Brasil). Para bloquear a peroxidase endógena, os cortes foram incubados em solução de peróxido de hidrogênio (Biotec, Curitiba, Brasil) e metanol 5% (Biotec, Curitiba, Brasil). Em seguida foi feita a recuperação antigênica com Imuno Retriver (Dako, Carpinteria, CA) em banho-maria à 99ºC por 30 min. Os cortes foram incubados com o anticorpo primário anti-MMP-8 monoclonal de coelho, clone: EP1252Y (Abcam, Cambridge, MA, EUA), a 4º C, overnight. A diluição do anticorpo em Antibody Diluent with Background-Reducing Components (DAKO Carpinteria, CA, código S302283) foi de 1:200. A detecção foi realizada com Advance link (Dako, Carpinteria, CA, código K406889), seguida pela Advance enzyme (Dako Corporation, Carpinteria, CA, código K406889) e o cromógeno 3,3' diaminobenzidina tetra hydrochloride (DAB - Sigma Chemical, St. Louis, código D7679). A contra-coloração foi realizada com solução de hematoxilina de Harris. Seguiu-se a desidratação (etanol) e a diafanização (xilol). Como controle positivo foi utilizado a placenta humana (2) enquanto como controle negativo omitiu-se o anticorpo primário.

Análise da Imunomarcação

Foram capturadas em cinco imagens aleatórias de cada lâmina através de uma microcâmera Dinolite® AM 423X (AmMo Eletronics Corporation, New Taipei city, Taiwan) acoplada ao microscópio Olympus BX-50 (Olympus, Tóquio, Japão) em magnificação de 400X. As imagens foram analisadas no programa de morfometria Image Pro-Plus 4.5 (Media Cybermetics, Silver Spring, EUA), que por segmentação semi-automatizada mensurou a área de MMP-8 em micrômetros quadrados. Foram consideradas imunopositivas as estruturas ou células coradas em marrom.

Análise Estatística

A análise estatística foi feita utilizando o software SPSS versão 22.0 (IBM, SPSS, Armonk, NY). Por meio do teste de Kolmogorov-Smirnov observou-se que

as variáveis apresentavam distribuição normal, optando-se então pelo teste ANOVA. Como o teste de homogenidade de variâncias de Levene revelou variâncias heterogêneas foi feito o teste de comparações múltiplas de Games Howell. O nível de significância adotado em todos os testes foi de 5%.

**Resultados**

Uma tendência a maior expressão de MMP-8 foi encontrada no grupo AF aos 14 dias quando comparado ao grupo C e o contrário foi observado aos 21 dias com significância estatística. Não foram observadas diferenças estatisticamente significativas entre os grupos aos 7 dias (Tabela 1, Figura 1).

TABELA 1: Valores médios e desvio padrão da área de MMP-8 ($\mu m^2$) nos grupos AF e C, nos tempos 7, 14 e 21 dias.

| Dias | AF (X±DP) | C (X±DP) | Teste de Games-Howell (valor de p) |
|---|---|---|---|
| 7 | 1,11± 1,22 | 1,34± 1,44 | 0,9997 |
| 14 | 4,21± 1,46 | 1,06± 1,32 | 0,0565 |
| 21 | 3,15± 0,62 | 5,63± 0,63 | 0,0021 |

Teste ANOVA a dois critérios: p= 0,0000; X= média; DP= desvio padrão
FONTE: pesquisa 2014.

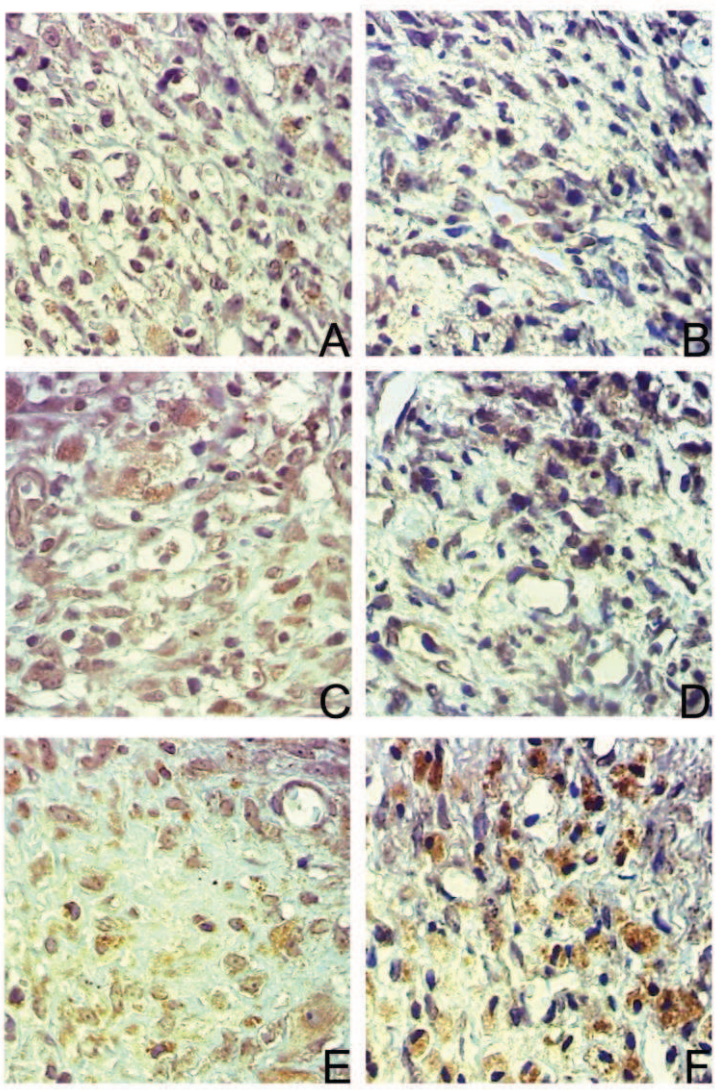



Figura 1- Imunoexpressão de MMP-8 (marrom) na região ulcerada aos **7 dias:** A) grupo AF e B) grupo C; **14 dias:** C) grupo AF e D) grupo C; **21 dias:** E) grupo AF e F) grupo C; (magnificação 400X).

## Discussão

Este é o primeiro estudo que avaliou a imunoexpressão da MMP-8 em úlceras induzidas no gastrocnêmico de ratos submetidos à atividade física, revelando que uma maior tendência expressão de MMP-8 foi encontrada no grupo AF aos 14 dias quando comparado ao grupo C e que o contrário foi observado aos 21 dias. Essa maior expressão de MMP-8 no grupo AF aos 14 dias foi compatível com uma menor área de colágeno tipo I observado em estudo prévio de Namba et al. (2014) (13). Aos 21 dias observou-se uma redução da MMP-8 comparando os grupos AF e C, o que possivelmente atribui-se a uma fadiga celular acarretando na diminuição dessa enzima, ou mesmo uma

regulação da sua produção por um mecanismo de feedback negativo para compensar sua maior liberação em período anterior. Entretanto essa diminuição da sua expressão no período de 21 dias ainda não foi suficiente para favorecer o aumento da deposição de colágeno tipo I, visto que no estudo prévio de Namba et al. (2014) (13), o grupo AF ainda apresentou menor porcentagem de colágeno tipo I nesse período. É possível que se esses animais fossem avaliados por um período de maior tempo, a expressão tanto de MMP-8, quanto a de colágeno tipo I, poderiam se equilibrar entre os grupos.

Essa tendência de superexpressão da MMP-8 observada aos 14 dias acarretando em uma menor deposição do colágeno tipo I (13) está em consonância com a literatura. Tal superexpressão está relacionada com a patogênese de feridas que não cicatrizam e com menor deposição de colágeno tipo I e essa enzima, quando em maior quantidade, degrada em maior proporção o colágeno tipo I, o que levaria uma menor área desse colágeno (4).

Na literatura até o presente momento, há uma carência de dados que retratam um modelo experimental semelhante ao do presente estudo. Por um lado isso trouxe dificuldades para comparar os achados, mas também valoriza os resultados aqui apresentados. Procurou-se comparar esses resultados com os achados da literatura em outros tipos de lesões e tecidos, mas tendo em comum a investigação da MMP-8 e atividade física. Neste estudo verificou-se que a atividade física modulou, aumentando em um primeiro momento e reduzindo posteriormente, a expressão da MMP-8 no gastrocnêmico de ratos, e essa modulação pela atividade física também foi verificada por Kadoglou et al (2013) (10), em ratos portadores de diabetes e com aterosclerose, uma vez que a atividade física com esteira por 6 semanas reduz a imunoexpressão de MMP-8 em placas ateroscleróticas, levando a uma diminuição da quebra do colágeno que leva a vulnerabilidade da placa esclerótica e sua progressão.

Continuando com a proposta de avaliar a modulação da MMP-8 pela atividade física, Abbott et al. (2012) (1) investigaram o potencial de exercícios vocais para modular a inflamação subsequente ao fonotrauma agudo e verificaram que os níveis de MMP-8 na secreção laríngea foi maior para fala espontânea, seguido pelo repouso vocal e menor para voz ressonante.

Ainda nessa linha de idéias, Paim et al. (2013) (14) e Schreiber et al. (2014) (16) avaliaram o efeito da atividade física Rúgbi, Basquete ou Handebol,

por no mínimo um ano em indivíduos com lesão na medula espinhal comparado com os sedentários. Foi verificado uma equivalência nos níveis séricos de MMP-8 em ambos os grupos, não havendo modulação desta enzima pelo exercício físico de forma oposta aos achados deste estudo.

Buscando comparar os achados da presente investigação com a literatura Pence et al. (2012) (15), demonstraram que o processo de reparo de feridas em pele foi maior com a atividade física (esteira), em ratos obesos. Porém, Namba et al. (2014) (13) demonstraram um atraso na deposição do colágeno tipo I, conferindo menor resistência a ferida e atrasando o processo de reparo. Esse atraso pode ser, ao menos parcialmente, atribuído à maior expressão da MMP-8 aos 14 dias observada no presente estudo. Outro aspecto que deve ser considerado para essas divergências com relação à reparo, seria que a úlcera na musculatura de ambos os estudos de Namba et al. (2014) (13) e o presente estudo poderia estar sendo constantemente traumatizada, pois ela estava localizada no grupo muscular requisitado pela atividade física, o que pode ter contribuído para esse atraso no processo de reparo.

A atividade física é de suma importância para atletas, mas pode resultar em lesões musculares quando os indivíduos são submetidos a treinamentos inadequados, excessivos ou ainda decorrentes das competições esportivas, o que leva a um afastamento do atleta das competições, bem como o fim precoce da carreira (3, 9, 11). Com base no conhecimento de que a atividade física (natação) desfavorece a deposição de colágeno tipo I (13), conferindo menor resistência à ferida, bem como altera a expressão da MMP-8, envolvida no processo de reparo, recomenda-se, dentro das limitações do presente estudo, o isolamento do grupo muscular lesionado, mantendo-o sem o treinamento físico até que o processo de reparo se complete.

## Conclusão

Os resultados sugerem que a atividade física altera, inicialmente aumentando e posteriormente diminuindo, a expressão da MMP-8, o que pode ter acarretado em uma menor deposição de colágeno tipo I aos 21 dias, observada em estudo prévio, o que confere menor resistência da ferida.

**Declaração de conflito de interesse:** Os autores declaram a inexistência de conflito de interesse real ou potencial.

## Referências


1 - Abbott KV, Li NY, Branski RC, Rosen CA, Grillo E, Steinhauer K, Hebda PA. Vocal exercise may attenuate acute vocal fold inflammation. *J Voice*. 2012;26(6):814.e1-13

2 - Anacker J, Segerer SE, Hagemann C, Feix S, Kapp M, Bausch R, Kämmerer U. Human decidua and invasive trophoblasts are rich sources of nearly all human matrix metalloproteinases*. Mol Hum Reprod*. 2011 Oct;17(10):637-52.

3 - De Moura NR, Borges LS, Santos VC, Joel GB, Bortolon JR, Hirabara SM, Cury-Boaventura MF, Pithon-Curi TC, Curi R, Hatanaka E. Muscle lesions and inflammation in futsal players according to their tactical positions. *J Strength Cond Res.* 2013;27(9):2612-8.

4 - Gajendrareddy PK, Engeland CG, Junges R, Horan MP, Rojas IG, Marucha PT. MMP-8 overexpression and persistence of neutrophils relate to stress-impaired healing and poor collagen architecture in mice. *Brain Behav Immun.* 2013;28:44-8.

5 - Gill SE, Parks WC. Metalloproteinases and their inhibitors: regulators of wound healing. *Int J Biochem Cell Biol*. 2008;40(6-7):1334-47.

6 - Gama YY, Grégio AM. Expressão de metaloproteinase-I em lesão muscular de ratos submetidos à atividade física [dissertation]. Curitiba (PR): Pontifícia Universidade Católica; 2013. 18p.

7 - Gonçalves AL, Luciano E. Inflammatory response in Wistar rats submitted to the physical activity. *Rev Bras Atividade Fís Saúde.* 1999;4:39-46.

8 - Hardy DC, Ross JH, Schuyler CA, Leite RS, Slate EH, Huang Y. Matrix metalloproteinase-8 expression in periodontal tissues surgically removed from diabetic and non-diabetic patients with periodontal disease. *J Clin Periodontol*. 2012;39(3):249-55.

9 - Horst K, Dienstknecht T, Sellei RM, Pape HC. Partial rupture of the hamstring muscle complex: a literature review on treatment options. *Eur J Orthop Surg Traumatol*. 2014;24(3):285-9.

10 - Kadoglou NP, Moustardas P, Kapelouzou A, Katsimpoulas M, Giagini A, Dede E, Kostomitsopoulos N, Karayannacos PE, Kostakis A, Liapis CD. The anti-inflammatory effects of exercise training promote atherosclerotic plaque stabilization in apolipoprotein E knockout mice with diabetic atherosclerosis. *Eur J Histochem*. 2013;57(1):e3.

11 - Malliaropoulos N, Papacostas E, Kiritsi O, Papalada A, Gougoulias N, Maffulli N. Posterior thigh muscle injuries in elite track and field athletes. *Am J Sports Med*. 2010;38(9):1813-9.

12 - Martins VL, Caley M, O'Toole EA. Matrix metalloproteinases and epidermal wound repair. *Cell Tissue Res*. 2013;351(2):255-68.

13 - Namba EL, Nawick TO, Thome MJ, Trevilatto PC, Machado MA, Azevedo-Alanis LR, Lima AA, Johann AC, Rosa EA, Ignácio AS, Grégio AM. Effect of Physical Activity on the Lesion Repair Process in the Gastrocnemius Muscle of Rats. *The Open Inflammation Journal*. 2014;(7):1-5.

14 - Paim LR, Schreiber R, Matos-Souza JR, Silva AA, Campos LF, Azevedo ER, Alonso K, de Rossi G, Etchebehere M, Gorla JI, Cliquet A Jr, Nadruz W Jr. Oxidized low-density lipoprotein, matrix-metalloproteinase-8 and carotid atherosclerosis in spinal cord injured subjects. *Atherosclerosis.* 2013;231(2):341-5.

15 - Pence BD, Dipietro LA, Woods JA. Exercise speeds cutaneous wound healing in high-fat diet-induced obese mice. *Med Sci Sports Exerc.* 2012;44(10):1846-54.

16 - Schreiber R, Paim LR, de Rossi G, Matos-Souza JR, Costa E Silva Ade A, Souza CM, Borges M, Azevedo ER, Alonso KC, Gorla JI, Cliquet A Jr, Nadruz W Jr. Matrix metalloproteinases and left ventricular function and structure in spinal cord injured subjects. Clin Chim Acta. 2014 Nov 1;437:136-40.

17 - Zornetto, N. L. Curso de Anatomia Humana. Terceira Ed. São Paulo: Instituto. Brasileiro de Edições Pedagógicas, 1979.

# ARTIGO EM INGLÊS

## TÍTULO: IMMUNOEXPRESSION OF METALLOPROTEINASE-8 IN MUSCLE INJURIES OF RATS SUBMITTED TO SWIM


**AUTHORS:**

1. Dáfine Zielinski
2. Aline Cristina Batista Rodrigues JOHANN, DDS, MSc, PhD[1]*

**INSTITUCIONAL AFFILIATIONS**

[1]Pontifícia Universidade Católica do Paraná, School of Health and Biosciences, Rua Imaculada Conceição, 1155, Prado Velho 80.215-901, Curitiba, PR, Brazil.

**Correspondent author**
Profa. Aline Cristina Batista Rodrigues Johann. PhD
Clínica de Odontologia
Pontifícia Universidade Católica do Paraná
Rua Imaculada Conceição, 1155, Prado Velho
80.215-901
Curitiba, PR, Brazil.
Telephone number: +55 41 3271-2592 Fax number: +55 41 3271-1405
E-mail: alinecristinabatista@yahoo.com.br

**Abstract**

Metalloproteinase 8 (MMP -8) is the predominant collagenase in the process of wound healing, which degrades type I collagen, the main type I collagen the wound tissue. Whereas physical activity interferes with wound healing process, the present study aimed to verify the immunoreactivity of MMP-8 in this process together with this activity. Forty-eight rats were divided into: a) PA- animals with muscle damage and had practiced physical activity (swim) with progressive loading; b) C- control with sedentary animals with muscle damage. Ulcers were induced chemically (NaOH 40%) in the gastrocnemius. On days 7, 14 and 21 after induction of muscle injury, the animals from both groups were killed. The lesion area was removed, histologically processed and submitted to immunohistochemistry using anti-MMP8. Five images of the sections, being evaluated by an analyzer software images. The PA ($4.21 \pm 1.46 \mu m^2$) group showed a trend toward increased expression of MMP- 8 at 14 days compared with C ($1.06 \pm 1.32 \mu m^2$; $p = 0.0565$), and the opposite was observed at 21 days: $3.15 \pm 0.62 \mu m^2$ to PA and $5.63 \pm 0.63 \mu m^2$ to C ( $p = 0.0021$). The results suggest that physical activity increase the expression of MMP-8 at 14 days and decrease at 21 days, which can be result in a lower deposition of type I collagen at 21 days observed in a previous study, which confers low wound strength.

***Key-words:*** Ulcer; Matrix Metalloproteinases; Exercise; Wound Healing

## Introduction

Metalloproteinases (MMPs) constitute a family of approximately 24 enzymes (endopeptidase) that is classified into five groups: collagenases [1, 8 and 13] , gelatinizes [2 and 9] , stromelysins [3, 10 , 11, 19] , such as the type membrane [14, 15, 16, 17], matrilisima [7 and 12], and others. Different among them structurally and functionally, are responsible for the degradation of the components of the extracellular matrix and basement membranes (5, 12).

The MMP-8, also called neutrophil collagenase, is an interstitial collagenase that cleaves collagen types I, II and III in fragments. Despite the nomenclature, MMP-8 is released by neutrophils, but also by other cellular types: fibroblasts, monocytes, macrophages, epithelial cells, endothelial cells and plasma cells in response to inflammatory stimuli (8). The MMP-8, the predominant collagenase in the wound healing process, degrades thetype I collagen, main type of collagen in wound tissue. The overexpression of this enzyme is related to the pathogenesis of non-healing wounds and with lesstype I collagen deposition in the wound and lower resistance of the scar (4).

Namba et al. (2014) (13) verified in rats with induced ulcer, lowertype I collagen deposition, at 14 and 21 days, comparing animals submitted to physical activity with sedentary. The authors concluded that the wound had less resistance in trained group at 21 days. In the same sample, Gama & Gregio (2013) (6) verified that the trained group showed a decrease of MMP- 1 expression at 14 days, and an increase at 21 days, concluding that the physical activity may change the MMP -1 expression, delaying the repair process of wounds at 14 days. Given these findings, it became necessary to assess whether MMP -8 expression, which is one of the enzymes responsible for the cleavage of thetype I collagen, could have influence in that lower deposition of type I collagen in the group submitted to physical activity.

The objective of this study was to determine the immunoexpression of MMP-8 in the process of repair of injured animals submitted to physical activity and sedentary animals.

## Material and Method

This study was approved by the Ethics Committee on Animal Use of the Pontifical Catholic University of Paraná (CEUA / PUCPR) under the protocol number 297/07. The sample was composed of previously processed blocks and stored in Experimental Pathology Laboratory of PUCPR (13). Forty eight adults male Wistar rats were used and divided into 2 groups: a) PA - animals with muscle damage and had practiced physical activity (swim) with progressive load; b) C - control with sedentary animals with muscle injury. Each group of animals was divided into three subgroups of 7, 14 and 21 days after induction of muscle injury.

The group C was submit to an aquatic environment, but promptly removed to mimic the trained group. Animals in the PA group were subjected to a swim scheme during the five days of the week and resting two days according to Gill & Luciano (1999) (7). Swim sessions started from 8:00 am and lasted 60 minutes. The animals were placed in an asbestos container (100 cm x 70 cm x 60 cm) water column of 40 cm at 32 °C.

The adaptation of the animals was carried out in a five-day period in the aquatic environment, increasing gradually training time (10 minutes daily). From the sixth day was attached to an elastic chest of the animal a charge of 5% of the total weight of the animal. The animals were weighed daily in order to adjust this load. After induction of ulcer on $11^{th}$ day, physical activity was continued somehow: 7 days, 60 minutes of swimming, totaling 18 days of physical activity ; 14 days, 60 minutes of swimming, totaling 25 days of physical activity ; 21 days, 60 minutes of swimming , totaling 32 days of physical activity.

After the animals being anesthetized with sodium thiopental (80 mg/ kg Critália, Itapira, Brazil), the ulcer was induced by subcutaneous injection of 0.08 ml of 40% NaOH in gastrocnemius of animals, where along the soleus muscle and long extensor digitorum if it forms the triceps sural and together, maintains a posture maintenance and make movements (17). At the end of the last training session, the animals were sacrificed by an overdose of sodium thiopental (150mg/ kg).

Immunohistochemistry reaction

The immunohistochemical reactions to MMP-8 protein were performed at the Experimental Pathology Laboratory of PUCPR. From paraffin blocks were obtained histological sections of 4 mm thick, which were deparaffinized (xylene, Biotec, Curitiba, Brazil) and hydrated (alcohol in decreasing solutions, Biotec, Curitiba, Brazil). To block endogenous peroxidase, the sections were incubated in hydrogen peroxide solution (Biotec, Curitiba, Brazil) and 5% methanol (Biotec, Curitiba, Brazil). Then was taken to antigen retrieval with Immuno Retriver (Dako, Carpinteria, CA) in a water bath at 99 °C for 30 min. Sections were incubated with the primary anti-MMP-8 rabbit monoclonal, clone: EP1252Y (Abcam, Cambridge, MA, USA) 4°C overnight. Dilution of the antibody in Antibody Diluent with Background Reducing Components, (DAKO Carpinteria, CA code S302283) was 1:200. Detection was performed with Advance link (Dako, Carpinteria, CA code K406889), followed by Advance enzyme (Dako Corporation, Carpinteria, CA code K406889), and the chromogen 3,3'-diaminobenzidine tetra hydrochloride (DAB - Sigma Chemical, St. Louis, D7679 code). Counter-staining was performed with Harris hematoxylin solution. Dehydration was followed (ethanol) and the diaphanization (xylene). As positive control was used the human placenta (2) as negative control as was omitted primary antibody.

Analysis of immunostaining

Random five images were captured of each slide through a microcamera Dinolite® AM 423X (Amino Electronics Corporation, New Taipei City, Taiwan) connected to an Olympus BX-50 microscope (Olympus, Tokyo, Japan) at a magnification of 400X. The images were analyzed with morphometry software Image Pro Plus 4.5 (Media Cybermetics, Silver Spring, USA), by semi-automated segmentation, that measured MMP-8 area in square micrometers. Were considered immunopositive the stained structures or cells in brown.

Statistical Analysis

Statistical analysis was performed using SPSS software version 22.0 (IBM, SPSS, Armonk, NY). By means of the Kolmogorov-Smirnov test was observed that normally distributed variables, choosing then the ANOVA test. As the

homogeneity variance test, Levene, revealed heterogeneous variances was performed the Games-Howell multiple comparison test. The significance level for all tests was 5%.

## Results

A tendency to increased expression of MMP-8 was found in the PA group at 14 days when compared to the C group and the opposite was observed at 21 days with statistical significance. No statistically significant differences were observed between the groups at 7 days (Table 1, Figure 1).

TABLE 1: Mean values and standard deviation of MMP-8 area ($\mu m^2$) in PA and C groups, at times 7, 14 and 21 days.

| Day | PA (X±DP) | C (X±DP) | Games-Howell test (p value) |
|---|---|---|---|
| 7 | 1.11± 1.22 | 1.34± 1.44 | 0.9997 |
| 14 | 4.21± 1.46 | 1.06± 1.32 | 0.0565 |
| 21 | 3.15± 0.62 | 5.63± 0.63 | 0.0021 |

Two-way ANOVA test*:* p= 0,0000; X= mean; DP= standard deviation
Source: Research 2014.

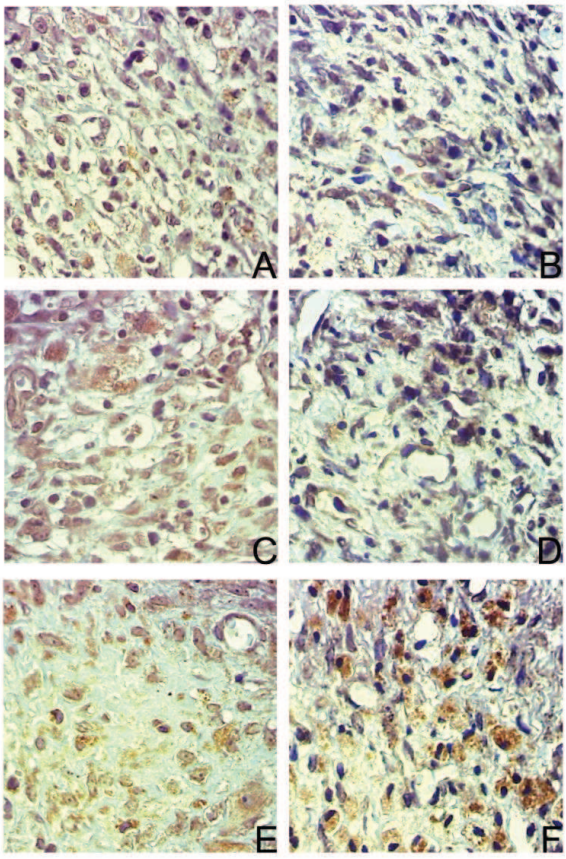



Figure 1- MMP-8 imunoexpression (brown) in ulcerated region at **7 days:** A) PA group and B) C group; **14 days:** C) PA group and D) C group; **21 days:** E) PA group and F) C group; (magnification, 400X).

## Discussion

This is the first study to assess the immunoreactivity of MMP-8 in induced ulcers in the gastrocnemius of rats submitted to physical activity, revealing that a greater tendency expression of MMP-8 was found in the PA group at 14 days compared to C group and the opposite was observed at 21 days. This increased expression of MMP-8 in the PA group at 14 days was consistent with a smaller area oftype I collagen observed in a previous study of Namba et al. (2014) (13). At 21 days there was a decrease of MMP-8 and C comparing PA groups, possibly assigned to fatigue cell resulting in decreased this enzyme or even a regulation of their production by a negative feedback mechanism to compensate its biggest release in the previous period. However, this decrease in expression in 21 days still was not enough to result in an increase in thetype I collagen deposition, because in the previous study of Namba et al. (2014) (13), the PA group yet showed a lower percentage oftype I collagen in this period. It is possible that if these animals were assessed for an upper period of time, the expression of both MMP-8, as thetype I collagen, it could be balanced between the groups.

This trend overexpression of MMP-8 observed at 14 days resulting in less deposition oftype I collagen (13) is consistent with the literature. Such overexpression is associated with the pathogenesis of non-healing wounds and less deposition of type I collagen and this enzyme in larger amounts, a higher proportion degrades type I collagen, that leads a smaller area of this collagen (4).

In the literature to date, there is a lack of data that show an experimental model similar to the present study. On the one hand it brought difficulties to compare the findings, but also values the results presented here. So, the results were comparing with the literature findings in other types of injuries and tissues but having in common the investigation of MMP-8 and physical activity. In this study it was found that physical activity modulated, increasing at first and subsequently reducing, the expression of MMP-8 in the gastrocnemius of rats and this modulation by physical activity was also observed by Kadoglou et al (2013) (10) in rats with diabetes and atherosclerosis, since physical activity in a mat for 6 weeks reduces the MMP-8 immunostaining of atherosclerotic plaques, leading to a decrease in the breakdown of collagen which leads to sclerotic plaque and the vulnerability of its progression.

Continuing with the purpose of evaluating the modulation of MMP-8 by physical activity, Abbott et al. (2012) (1) investigated the potential of vocal exercises to modulate inflammation subsequent to acute traumatic origin and found that MMP-8 levels in laryngeal secretion was higher for spontaneous speech, followed by vocal rest and lower for resonant voice.

Although this line of ideas, Paim et al. (2013) (14) and Schreiber et al. (2014) (16) evaluated the effect of physical activity Rugby, Basketball or Handball for at least one year, in individuals with spinal cord injury compared with sedentary. Equivalence in serum MMP-8 was observed in both groups, with no modulation of this enzyme by physical exercise in the opposite way to the findings of the present study.

Searching to compare the discoveries of the presented research with the literature, Pence et al (2012) (15) demonstrated that the process of wound repair in skin was higher with the physical activity (treadmill) in obese rats. However, Namba et al. (2014) (13) showed a delay in the deposition of type I collagen, giving less resistance and delaying the wound repair process. This delay can be at least partially attributed to the increased expression of MMP-8 to 14 days observed in this study. Another aspect that should be considered for these differences with respect to repair, would be the ulcer in the muscles of both studies Namba et al. (2014) (13) and the present study could have been constantly traumatized because it was located in the muscle group requested by physical activity, which may have contributed to the delay in the repair process.

Physical activity is very important for athletes, but can result in muscle damage when individuals are subjected to inadequate training, excessive or resulting from sports competitions, which leads to an athlete's departure from the competitions as well as early end of career (3, 9, 11). Based on the knowledge that physical activity (swim) disfavors the deposition of type I collagen (13), providing less resistance to the wound, and alters the expression of MMP-8, involved in the repair process, it is recommended, in the limitations of this study, the isolation of the injured muscle group, keeping it without physical training until the healing process is complete.

## Conclusion

The results suggest that physical activity modifies, in beginning increase and after decreasing, the expression of MMP-8, which can be cause a lower deposition of type I collagen at 21 days that was observed in a previous study giving lower strength of the wound.

**References**


1 - Abbott KV, Li NY, Branski RC, Rosen CA, Grillo E, Steinhauer K, Hebda PA. Vocal exercise may attenuate acute vocal fold inflammation. *J Voice*. 2012;26(6):814.e1-13

2 - Anacker J, Segerer SE, Hagemann C, Feix S, Kapp M, Bausch R, Kämmerer U. Human decidua and invasive trophoblasts are rich sources of nearly all human matrix metalloproteinases. *Mol Hum Reprod*. 2011 Oct;17(10):637-52.

3 - De Moura NR, Borges LS, Santos VC, Joel GB, Bortolon JR, Hirabara SM, Cury-Boaventura MF, Pithon-Curi TC, Curi R, Hatanaka E. Muscle lesions and inflammation in futsal players according to their tactical positions. *J Strength Cond Res.* 2013;27(9):2612-8.

4 - Gajendrareddy PK, Engeland CG, Junges R, Horan MP, Rojas IG, Marucha PT. MMP-8 overexpression and persistence of neutrophils relate to stress-impaired healing and poor collagen architecture in mice. *Brain Behav Immun.* 2013;28:44-8.

5 - Gill SE, Parks WC. Metalloproteinases and their inhibitors: regulators of wound healing. *Int J Biochem Cell Biol*. 2008;40(6-7):1334-47.

6 - Gama YY, Grégio AM. Expressão de metaloproteinase-I em lesão muscular de ratos submetidos à atividade física [dissertation]. Curitiba (PR): Pontifícia Universidade Católica; 2013. 18p.

7 - Gonçalves AL, Luciano E. Inflammatory response in Wistar rats submitted to the physical activity. *Rev Bras Atividade Fís Saúde.* 1999;4:39-46.

8 - Hardy DC, Ross JH, Schuyler CA, Leite RS, Slate EH, Huang Y. Matrix metalloproteinase-8 expression in periodontal tissues surgically removed from diabetic and non-diabetic patients with periodontal disease. *J Clin Periodontol*. 2012;39(3):249-55.

9 - Horst K, Dienstknecht T, Sellei RM, Pape HC. Partial rupture of the hamstring muscle complex: a literature review on treatment options. *Eur J Orthop Surg Traumatol*. 2014;24(3):285-9.

10 - Kadoglou NP, Moustardas P, Kapelouzou A, Katsimpoulas M, Giagini A, Dede E, Kostomitsopoulos N, Karayannacos PE, Kostakis A, Liapis CD. The anti-inflammatory effects of exercise training promote atherosclerotic plaque stabilization in apolipoprotein E knockout mice with diabetic atherosclerosis. *Eur J Histochem*. 2013;57(1):e3.

11 - Malliaropoulos N, Papacostas E, Kiritsi O, Papalada A, Gougoulias N, Maffulli N. Posterior thigh muscle injuries in elite track and field athletes. *Am J Sports Med*. 2010;38(9):1813-9.

12 - Martins VL, Caley M, O'Toole EA. Matrix metalloproteinases and epidermal wound repair. *Cell Tissue Res*. 2013;351(2):255-68.

13 - Namba EL, Nawick TO, Thome MJ, Trevilatto PC, Machado MA, Azevedo-Alanis LR, Lima AA, Johann AC, Rosa EA, Ignácio AS, Grégio AM. Effect of Physical Activity on the Lesion Repair Process in the Gastrocnemius Muscle of Rats. *The Open Inflammation Journal*. 2014;(7):1-5.

14 - Paim LR, Schreiber R, Matos-Souza JR, Silva AA, Campos LF, Azevedo ER, Alonso K, de Rossi G, Etchebehere M, Gorla JI, Cliquet A Jr, Nadruz W Jr. Oxidized low-density lipoprotein, matrix-metalloproteinase-8 and carotid atherosclerosis in spinal cord injured subjects. *Atherosclerosis.* 2013;231(2):341-5.

15 - Pence BD, Dipietro LA, Woods JA. Exercise speeds cutaneous wound healing in high-fat diet-induced obese mice. *Med Sci Sports Exerc.* 2012;44(10):1846-54.

16 - Schreiber R, Paim LR, de Rossi G, Matos-Souza JR, Costa E Silva Ade A, Souza CM, Borges M, Azevedo ER, Alonso KC, Gorla JI, Cliquet A Jr, Nadruz W Jr. Matrix metalloproteinases and left ventricular function and structure in spinal cord injured subjects. Clin Chim Acta. 2014 Nov 1;437:136-40.

17 - Zornetto, N. L. Course of Human Anatomy. Third Ed Sao Paulo: Institute Brazilian Pedagogical Issues , 1979.

ANEXOS

# ANEXO A- Parecer de comitê de ética

PUCPR

**PONTIFÍCIA UNIVERSIDADE CATÓLICA DO PARANÁ**

NÚCLEO DE BIOÉTICA
COMITÊ DE ÉTICA NO USO DE ANIMAIS

**PARECER DE PROTOCOLO DE PESQUISA**

**Parecer nº:** 221/07 CEUA PUCPR
**Registro do projeto no CEUA:** 297

**Data do parecer:** 04/12/2007

**Título do Projeto:**
Análise do processo de reparo em lesão bucal e muscular de ratos submetidos a exercícios físicos.

**Pesquisador responsável:**
Ana Maria Trindade Grégio

**Equipe da pesquisa:**
Eli Luis Namba; Mariele Thomé Jung; Rodrigo Bertier Valentim

**Instituição:**
PUCPR

**Categoria do Experimento - Categoria B**

| Espécie de Animal | Sexo | Idade ou peso | Quantidade |
|---|---|---|---|
| Rattus norvrgicus albinus, rodentia, mammalia da linhagem wistar | Macho | de 45 a 50dias, peso de 150g. | 166 |

O colegiado do CEUA em reunião no dia 29/11/2007, avaliou o projeto e emite o seguinte parecer: **APROVADO.**

Eventuais modificações ou emendas ao protocolo devem ser apresentadas ao CEUA-PUCPR de forma clara e sucinta, identificando a parte do protocolo a ser modificado e as suas justificativas.

Se a pesquisa, ou parte dela for realizada em outras instituições, cabe ao pesquisador não iniciá-la antes de receber a autorização formal para a sua realização. O documento que autoriza o início da pesquisa deve ser carimbado e assinado pelo responsável da instituição e deve ser mantido em poder do

pesquisador responsável, podendo ser requerido por este CEUA em qualquer tempo.

Lembramos ao senhor pesquisador que é obrigatório encaminhar o relatório anual parcial e relatório final da pesquisa a este CEUA.

Curitiba, 4 de dezembro de 2007.

Comitê de Ética no Uso de Animais - CEUA
PUCPR

Atenciosamente

Profa. Gracinda Maria D'Almeida e Oliveira
Coordenadora do CEUA
PUC PR

1

2

# 1 ANEXO B- Análise estatística

**Tests of Normality**

| Grupo x Tempo | | Kolmogorov-Smirnov[a] | | | Shapiro-Wilk | | |
|---|---|---|---|---|---|---|---|
| | | Statistic | Df | Valor p | Statistic | df | Valor p |
| MMP-8 (µm2) | Sedentários / 7 dias | ,308 | 5 | ,138 | ,831 | 5 | ,141 |
| | Sedentários / 14 dias | ,331 | 5 | ,076 | ,781 | 5 | ,056 |
| | Sedentários / 21 dias | ,218 | 5 | ,200* | ,920 | 5 | ,531 |
| | Treinados / 7 dias | ,229 | 5 | ,200* | ,869 | 5 | ,264 |
| | Treinados / 14 dias | ,330 | 5 | ,080 | ,801 | 5 | ,082 |
| | Treinados / 21 dias | ,223 | 5 | ,200* | ,966 | 5 | ,850 |

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

**Descriptives**

MMP-8 (µm2)

| | N | Mean | Std. Deviation | Std. Error | 95% Confidence Interval for Mean | | Minimum | Maximum |
|---|---|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound | | |
| Sedentários | 15 | 2,68 | 2,43 | 0,63 | 1,34 | 4,02 | 0,02 | 6,33 |
| Treinados | 15 | 2,82 | 1,71 | 0,44 | 1,88 | 3,77 | 0,02 | 5,44 |
| Total | 30 | 2,75 | 2,06 | 0,38 | 1,98 | 3,52 | 0,02 | 6,33 |

**Test of Homogeneity of Variances**

MMP-8 (µm2)

| Levene Statistic | df1 | df2 | Valor p |
|---|---|---|---|
| 3,380 | 1 | 28 | 0,0766 |

**Descriptives**

MMP-8 (µm2)

| | N | Mean | Std. Deviation | Std. Error | 95% Confidence Interval for Mean | | Minimum | Maximum |
|---|---|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound | | |
| 7 dias | 10 | 1,23 | 1,27 | 0,40 | 0,32 | 2,13 | 0,02 | 3,13 |
| 14 dias | 10 | 2,64 | 2,11 | 0,67 | 1,12 | 4,15 | 0,02 | 5,44 |
| 21 dias | 10 | 4,39 | 1,43 | 0,45 | 3,36 | 5,42 | 2,37 | 6,33 |
| Total | 30 | 2,75 | 2,06 | 0,38 | 1,98 | 3,52 | 0,02 | 6,33 |

**Test of Homogeneity of Variances**

MMP-8 (µm2)

| Levene Statistic | df1 | df2 | Valor p |
|---|---|---|---|
| 1,100 | 2 | 27 | 0,3472 |

**Descriptives**

MMP-8 (µm2)

| | N | Mean | Std. Deviation | Std. Error | 95% Confidence Interval for Mean | | Minimum | Maximum |
|---|---|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound | | |
| Sedentários / 7 dias | 5 | 1,34 | 1,44 | 0,64 | -0,45 | 3,13 | 0,02 | 3,13 |
| Sedentários / 14 dias | 5 | 1,06 | 1,32 | 0,59 | -0,57 | 2,70 | 0,02 | 2,73 |
| Sedentários / 21 dias | 5 | 5,63 | 0,63 | 0,28 | 4,85 | 6,41 | 4,89 | 6,33 |
| Treinados / 7 dias | 5 | 1,11 | 1,22 | 0,55 | -0,41 | 2,63 | 0,02 | 2,72 |
| Treinados / 14 dias | 5 | 4,21 | 1,46 | 0,65 | 2,39 | 6,02 | 2,36 | 5,44 |
| Treinados / 21 dias | 5 | 3,15 | 0,62 | 0,28 | 2,38 | 3,91 | 2,37 | 4,01 |
| Total | 30 | 2,75 | 2,06 | 0,38 | 1,98 | 3,52 | 0,02 | 6,33 |

**Test of Homogeneity of Variances**

MMP-8 (µm2)

| Levene Statistic | df1 | df2 | Valor p |
|---|---|---|---|
| 5,299 | 5 | 24 | 0,0020 |

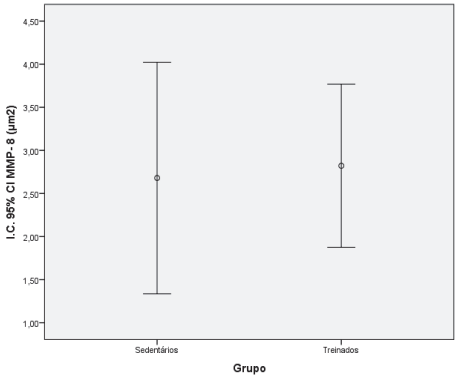
**Tests of Between-Subjects Effects**

Dependent Variable: MMP-8 (µm2)

| Source | Type III Sum of Squares | Df | Mean Square | F | Valor p | Observed Power[b] |
|---|---|---|---|---|---|---|
| Grupo | 0,1506 | 1 | 0,1506 | 0,1099 | 0,743084 | 0,061702 |
| Tempo | 50,2366 | 2 | 25,1183 | 18,3417 | 0,000015 | 0,999598 |
| Grupo * Tempo | 40,1126 | 2 | 20,0563 | 14,6454 | 0,000070 | 0,997114 |
| Error | 32,8671 | 24 | 1,3695 | | | |
| Corrected Total | 123,3668 | 29 | | | | |

a. R Squared = ,734 (Adjusted R Squared = ,678)

b. Computed using alpha = ,05



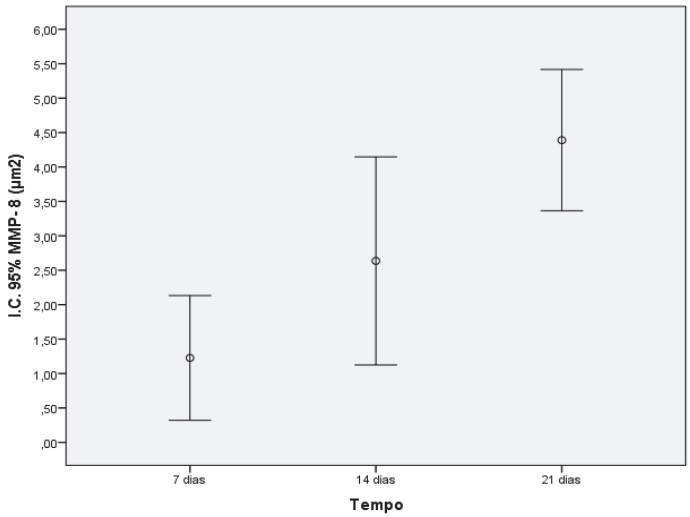

**Multiple Comparisons**

Dependent Variable: MMP-8 (µm2)

Tukey HSD

| (I) Tempo | | Mean Difference (I-J) | Std. Error | Valor p | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| 7 dias | 14 dias | -1,4096 | 0,7360 | 0,1538 | -3,2345 | 0,4152 |
| | 21 dias | -3,16351* | 0,7360 | 0,0006 | -4,9884 | -1,3386 |
| 14 dias | 7 dias | 1,4096 | 0,7360 | 0,1538 | -0,4152 | 3,2345 |
| | 21 dias | -1,7539 | 0,7360 | 0,0614 | -3,5788 | 0,0710 |
| 21 dias | 7 dias | 3,16351* | 0,7360 | 0,0006 | 1,3386 | 4,9884 |
| | 14 dias | 1,7539 | 0,7360 | 0,0614 | -0,0710 | 3,5788 |

*. The mean difference is significant at the 0.05 level.

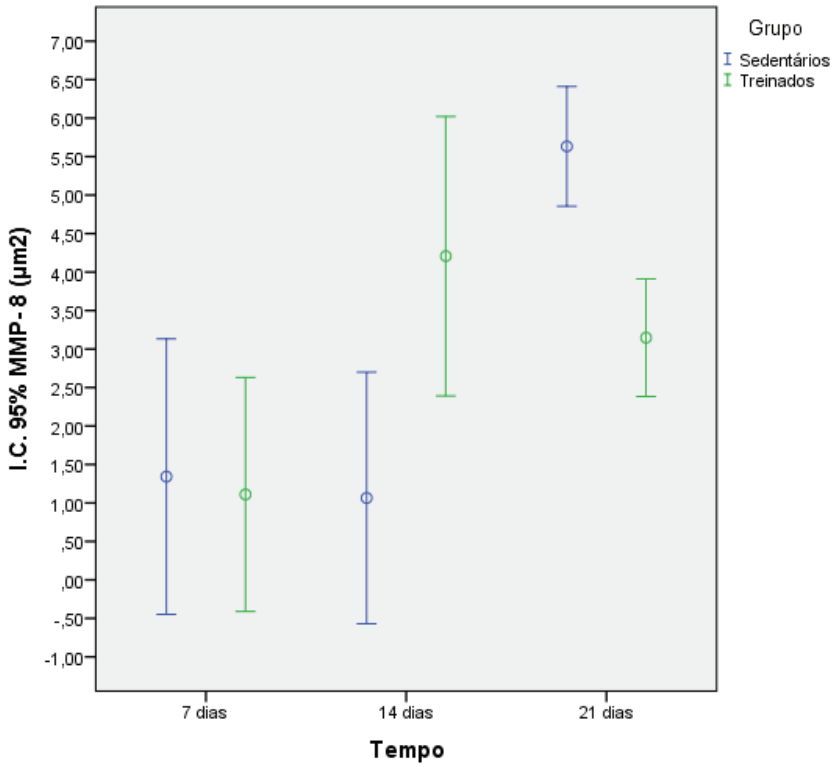


**Multiple Comparisons**

Dependent Variable: MMP-8 (µm2)
Games-Howell

| (I) Grupo x Tempo | | Mean Difference (I-J) | Std. Error | Valor p | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| Sedentários / 7 dias | Sedentários / 14 dias | 0,2773 | 0,8728 | 0,9994 | -2,9183 | 3,4729 |
| | Sedentários / 21 dias | -4,29047* | 0,7027 | 0,0089 | -7,1842 | -1,3968 |
| | Treinados / 7 dias | 0,2316 | 0,8456 | 0,9997 | -2,8785 | 3,3417 |
| | Treinados / 14 dias | -2,8649 | 0,9180 | 0,1017 | -6,2193 | 0,4894 |
| | Treinados / 21 dias | -1,8049 | 0,7007 | 0,2465 | -4,7004 | 1,0905 |
| Sedentários / 14 dias | Sedentários / 7 dias | -0,2773 | 0,8728 | 0,9994 | -3,4729 | 2,9183 |
| | Sedentários / 21 dias | -4,56778* | 0,6520 | 0,0039 | -7,2062 | -1,9293 |
| | Treinados / 7 dias | -0,0457 | 0,8040 | 1,0000 | -2,9871 | 2,8958 |
| | Treinados / 14 dias | -3,1422 | 0,8798 | 0,0565 | -6,3659 | 0,0814 |
| | Treinados / 21 dias | -2,0822 | 0,6498 | 0,1224 | -4,7214 | 0,5569 |
| Sedentários / 21 dias | Sedentários / 7 dias | 4,29047* | 0,7027 | 0,0089 | 1,3968 | 7,1842 |
| | Sedentários / 14 dias | 4,56778* | 0,6520 | 0,0039 | 1,9293 | 7,2062 |
| | Treinados / 7 dias | 4,52210* | 0,6151 | 0,0026 | 2,0686 | 6,9756 |
| | Treinados / 14 dias | 1,4255 | 0,7114 | 0,4397 | -1,5121 | 4,3632 |
| | Treinados / 21 dias | 2,48555* | 0,3927 | 0,0021 | 1,0505 | 3,9206 |
| Treinados / 7 dias | Sedentários / 7 dias | -0,2316 | 0,8456 | 0,9997 | -3,3417 | 2,8785 |
| | Sedentários / 14 dias | 0,0457 | 0,8040 | 1,0000 | -2,8958 | 2,9871 |
| | Sedentários / 21 dias | -4,52210* | 0,6151 | 0,0026 | -6,9756 | -2,0686 |
| | Treinados / 14 dias | -3,0966 | 0,8528 | 0,0535 | -6,2371 | 0,0440 |
| | Treinados / 21 dias | -2,0366 | 0,6128 | 0,1037 | -4,4898 | 0,4167 |
| Treinados / 14 dias | Sedentários / 7 dias | 2,8649 | 0,9180 | 0,1017 | -0,4894 | 6,2193 |
| | Sedentários / 14 dias | 3,1422 | 0,8798 | 0,0565 | -0,0814 | 6,3659 |
| | Sedentários / 21 dias | -1,4255 | 0,7114 | 0,4397 | -4,3632 | 1,5121 |
| | Treinados / 7 dias | 3,0966 | 0,8528 | 0,0535 | -0,0440 | 6,2371 |
| | Treinados / 21 dias | 1,0600 | 0,7094 | 0,6817 | -1,8795 | 3,9995 |
| Treinados / 21 dias | Sedentários / 7 dias | 1,8049 | 0,7007 | 0,2465 | -1,0905 | 4,7004 |
| | Sedentários / 14 dias | 2,0822 | 0,6498 | 0,1224 | -0,5569 | 4,7214 |
| | Sedentários / 21 dias | -2,48555* | 0,3927 | 0,0021 | -3,9206 | -1,0505 |
| | Treinados / 7 dias | 2,0366 | 0,6128 | 0,1037 | -0,4167 | 4,4898 |
| | Treinados / 14 dias | -1,0600 | 0,7094 | 0,6817 | -3,9995 | 1,8795 |

*. The mean difference is significant at the 0.05 level.


Grupo
Sedentários
Treinados
I.C. 95% MMP-8 (µm2)
7,00
6,50
6,00
5,50
5,00
4,50
4,00
3,50
3,00
2,50
2,00
1,50
1,00
,50
,00
-,50
-1,00
7 dias
14 dias
21 dias
Tempo

1

2

**ANEXO C- Normas para publicação**

**Medicine & Science in Sports & Exercise®**
**Online Submission and Review System**

## Information for Authors

### 1. General Information

*Medicine & Science in Sports & Exercise*® (MSSE®) is the official journal of the American College of Sports Medicine and is published monthly. Manuscripts dealing with original investigations, clinical studies, special communications, or brief reviews on topics relevant to the areas of interest of the College will be considered for publication.

Membership in the American College of Sports Medicine is not a requisite for publication in the journal, nor does it influence editorial decisions. The journal is owned by the American College of Sports Medicine and is copyrighted for the protection of authors and the College.

### Address for Correspondence

Medicine & Science in Sports & Exercise , 401 West Michigan Street, Indianapolis, IN 46202-3233 USA, Phone: 317-634-8932, Fax: 317-634-8927, msse@acsm.org.

### Submission Types

In addition to original investigations, the journal publishes

- Clinical Investigations & Case Studies
- Brief Reviews
- Symposium Proceedings
- Special Communications
    - Methodological Advances
    - Letters to the Editor-in-Chief
    - Book Reviews

***Clinical Investigations & Case Studies***

Authors may submit manuscripts describing specific clinical cases that provide relevant information on diagnosis and therapy of a particular case that proves unique to clinical sports medicine. Manuscripts should be current, concise, accurate, understandable, and contain the following:

- An abstract that contains the clinical implications.
- An introduction that provides commentary with regard to the clinical problem, which will be explained using the case as an example. It is important to document the patient's agreement to the use of their clinical data in the presentation.
- A brief case report including history, physical examination, and laboratory findings followed by treatment and outcome.
- A discussion section that explains in detail the clinical implications over the course of the case as well as key aspects of the case that may be unique or may differ from similar reported cases in the medical literature.

***Brief Reviews***

Brief review articles (maximum 25 double-spaced pages, including references—limit 75) will be screened by the Editor-in-Chief before entering the review process. Authors of review articles shall be established, recognized experts in the field. Literature reviews in conjunction with collegiate thesis work are not acceptable review articles.

***Symposium Proceedings***

Submission of ACSM Annual Meeting symposia papers is by Editor-in-Chief invitation only. Symposia papers from any ACSM Annual Meeting must be received in the Editorial Office before December 1 of the year of presentation. Previously stated submission requirements shall be followed; however, presentations should not exceed 20 typewritten, double-spaced pages. Authors who use previously published material shall obtain prior written permission to reprint from the publisher holding the copyright and provide a quality original for publication. (See "Previously Published Material.") All invited symposia

manuscripts are subject to the peer-review process. Organizers of symposia concerned with new developments in sports medicine and exercise science are encouraged to contact the Editor-in-Chief regarding the possibility of publication.

***Special Communications***

**Methodological Advances**

Manuscripts that deal with new methods, important modifications of existing ones, or applications of new equipment will be considered for publication in a section titled "Methodological Advances." Authors are strongly encouraged to familiarize themselves with the recently published articles in *Medicine & Science in Sports & Exercise*®, as the journal will not consider for publication those manuscripts that present results of articles previously published.

**Letters to the Editor-in-Chief**

Letters addressed to the Editor-in-Chief will be considered for publication if they promote intellectual discussion of an *MSSE*® article published within the previous 12 months. Letters should contain an informative title and follow the submission requirements for manuscripts. Letters are limited to 500 words and a maximum of eight (8) references. If the letter is accepted for publication, a copy will be sent to the author of the original article with an invitation to submit a rebuttal that will be published with the letter. Letter responses will be held to the same length and reference requirements.

**Books for Review**

ACSM is pleased to provide readers with the most current reviews of just released publications from Doody Enterprises, Inc. and, therefore, does not accept books from publishers or authors for the purpose of independent review.

**Authorship**

To be an author, each individual shall have contributed to the manuscript in at least two (2) of the following areas:

- Significant manuscript writer
- Significant manuscript reviewer/reviser
- Concept and design
- Data acquisition
- Data analysis and interpretation
- Statistical expertise

For additional authorship details, see “Uniform Requirements for Manuscripts Submitted to Biomedical Journals” at http://www.icmje.org/.

**Dual Submissions**

Manuscripts are considered for publication on the condition that they are contributed solely to this journal and, therefore, have not been and will not be published elsewhere, in part or in whole. Manuscripts containing data that have been posted to the Internet for public access will not be considered for publication.

**Conflicts of Interest**

Authors must state all possible conflicts of interest in the manuscript, including financial, consultant, institutional, and other relationships that might lead to bias or a conflict of interest. If there is no conflict of interest, this should also be explicitly stated as none declared. All sources of funding should be acknowledged in the manuscript. All relevant conflicts of interest and sources of funding should be included after the acknowledgments section of the manuscript with the heading "Conflicts of Interest and Source of Funding". For example:

Conflicts of Interest and Source of Funding: A has received honoraria from Company Z. B is currently receiving a grant (#12345) from Organization Y, and is on the speaker's bureau for Organization X – the CME organizers for Company A. For the remaining authors none were declared.

## Misconduct

Fabrication, falsification, plagiarism, or any other practice by an author that seriously deviates from practices commonly accepted in the discipline or in the academic and research communities generally for proposing, conducting, or reporting research and creative activities will be considered actionable misconduct. The act of serious deviation includes duplicate publication or unethical use of research participants. Misconduct does not include honest error or honest differences in the interpretation or judgment of data.

If there is a suspicion or allegation of misconduct directed toward any author of a work submitted or published by *MSSE*®, the journal reserves the right to a) forward relevant material to the appropriate authorities at the author's institution/employer for investigation, and/or b) conduct an independent investigation. If an author is found to have committed misconduct, *MSSE*® reserves the right to debar the author, either temporarily or permanently, from publishing in *MSSE*® and/or all journals associated with ACSM. *MSSE*®'s Editor-in-Chief, in consultation with the ACSM Publications Committee, will impose debarment or any lesser sanction found to be appropriate in light of the misconduct finding. Depending on the nature of the misconduct finding, a retraction of the author's relevant paper(s) published in *MSSE*® may occur and notification of the retraction may be published in a future issue.

For additional misconduct details, see "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" at http://www.icmje.org/.

## Reproduction of Text and Figures

*Medicine & Science in Sports & Exercise*® is a copyrighted publication. It is official policy of the journal that single copies of figures can be used as slides or overhead projector transparencies if the purpose is for educational matters. Authors submitting manuscripts and publishing articles in this journal should be aware of this policy and must accept the provisions described. Persons making such reproductions should include an appropriate bibliographical citation on the slide or transparency. This policy does not include the reproduction and use of

such figures in printed form, especially in regard to inclusion in textbooks. Permission to reproduce the text portion of any article in whole or in part must be obtained from both the senior author of the particular article and the publisher, Lippincott Williams & Wilkins, 351 West Camden Street, Baltimore, MD 21201-2436 USA (http://www.lww.com/resources/permissions/index.html).

**Previously Published Material**

*Medicine & Science in Sports & Exercise*® will accept only original, unpublished illustrations and tables, except in the cases of review articles, symposia, and meta-analyses. Authors of review articles, symposia, and meta-analyses papers who do use previously published material shall obtain prior written permission to reprint from the publisher holding the copyright and be able to provide a quality original to the Editorial Office for publication. It also is customary that written permission from the original authors be requested and received. The statement "used by permission" must appear in the caption of the figure or table with complete reference citation. Permission to reprint, if required, must accompany the manuscript at the time of submission.

**Permissions**

For permission and/or rights to use content for which the copyright holder is LWW or the society, please go to the journal's website and after clicking on the relevant article, click on the "Request Permissions" link under the "Article Tools" box that appears on the right side of the page. Alternatively, send an e-mail to customercare@copyright.com.

For Translation Rights & Licensing queries, contact Silvia Serra, Translations Rights, Licensing & Permissions Manager, Wolters Kluwer Health (Medical Research) Ltd, 250 Waterloo Road, London SE1 8RD, UK. Phone: +44 (0) 207 981 0600. E-mail: silvia.serra@wolterskluwer.com

For Special Projects and reprints (U.S./Canada), contact Alan Moore, Director of Sales, Lippincott Williams & Wilkins, Two Commerce Square, 2001 Market Street, Philadelphia, PA 19103. Phone: 215-521-8638. E-mail: alan.moore@wolterskluwer.com

For Special Projects and Reprints (non-U.S./Canada), contact Silvia Serra, Translations Rights, Licensing & Permissions Manager, Wolters Kluwer Health (Medical Research) Ltd, 250 Waterloo Road, London SE1 8RD, UK. Phone: +44 (0) 207 981 0600. E-mail: silvia.serra@wolterskluwer.com

**ACSM Manuscripts & NIH Public Access Policy *Overview***

Since April 7, 2008, the National Institutes of Health (NIH) has required "all investigators funded by the NIH [to] submit, or have submitted for them to the National Library of Medicine's PubMed Central an electronic version of their final peer-reviewed manuscripts upon acceptance for publication, to be made publicly available no later than 12 months after the official date of publication..."

***Lippincott Williams & Wilkins Submission Service***

To assist our authors in meeting the requirements of the NIH and certain other funding bodies (Wellcome Trust and Howard Hughes Medical Institute), Lippincott Williams & Wilkins will transmit the accepted manuscript, shortly after acceptance, to PubMed Central (PMC) on their behalf. Seehttp://journals.lww.com/acsm-msse/_layouts/oaks.journals/nih.aspx for additional details.

**Open Access**

LWW's hybrid open access option is offered to authors whose articles have been accepted for publication. With this choice, articles are made freely available online immediately upon publication. Authors may take advantage of the open access option at the point of acceptance to ensure that this choice has no influence on the peer review and acceptance process. These articles are subject to the journal's standard peer-review process and will be accepted or rejected based on their own merit. Those individuals making the decision to accept or reject an article are not aware if the article is a funded article or has been selected for open access.

Authors of accepted peer-reviewed articles have the choice to pay a fee to allow perpetual unrestricted online access to their published article to readers globally, immediately upon publication. The article processing charge for *Medicine & Science in Sports & Exercise* is $3,000. The article processing charge for authors

funded by the Research Councils UK (RCUK) is $3,800. The publication fee is charged on acceptance of the article and should be paid within 30 days by credit card by the author, funding agency or institution. Payment must be received in full for the article to be published open access.

***Authors Retain Copyright***

Authors retain their copyright for all articles they opt to publish open access. Authors grant LWW a license to publish the article and identify itself as the original publisher.

***Creative Commons License***

Articles opting for open access will be freely available to read, download, and share from the time of publication. Articles are published under the terms of the Creative Commons License Attribution-NonCommerical No Derivative 3.0, which allows readers to disseminate and reuse the article, as well as share and reuse of the scientific material. It does not permit commercial exploitation or the creation of derivative works without specific permission. To view a copy of this license visit: http://creativecommons.org/licenses/by-nc-nd/3.0.

***Compliance with NIH, RCUK, Wellcome Trust, and Other Research Funding Agency Accessibility Requirements***

A number of research funding agencies now require or request authors to submit the post-print (the article after peer review and acceptance but not the final published article) to a repository that is accessible online by all without charge. As a service to our authors, LWW identifies to the National Library of Medicine (NLM) articles that require deposit and transmits the post-print of an article based on research funded in whole or in part by the National Institutes of Health, Howard Hughes Medical Institute, or other funding agencies to PubMed Central. The revised Copyright Transfer Agreement provides the mechanism. LWW ensures that authors can fully comply with the public access requirements of major funding bodies worldwide. Additionally, all authors who choose the open access option will have their final published article deposited into PubMed Central.

RCUK-funded authors can choose to publish their paper as open access with the payment of an article process charge, or opt for their accepted manuscript to be deposited (green route) into PMC with an embargo.

With both the gold and green open access options, the author will continue to sign the Copyright Transfer Agreement (CTA) as it provides the mechanism for LWW to ensure that the author is fully compliant with the requirements. After signature of the CTA, the author will then sign a License to Publish where they will then own the copyright.

It is the responsibility of the author to inform the Editorial Office and/or LWW that they have RCUK funding. LWW will not be held responsible for retroactive deposits to PMC if the author has not completed the proper forms.

***FAQ for Open Access***

http://links.lww.com/LWW-ES/A48

**2. Manuscript Submission Requirements and Preparation**

*Manuscripts that do not comply with the following requirements and directives for process, style, and format will not enter the peer-review process and will be returned to the author.*

***Language***

English is the language of the publication. Authors who speak English as a second language are encouraged to seek the assistance of a colleague experienced in writing for English language journals.

Use of the terms “gender" and "sex” should comply with the definitions used by the World Health Organization (http://www.who.int/gender/whatisgender/en/) as follows:

- “Sex” refers to the biological and physiological characteristics that define men and women.
- “Gender” refers to the socially constructed roles, behaviors, activities, and attributes that a given society considers appropriate for men and women.

Authors are encouraged to use nonsexist language as defined by the American Psychological Association (American Psychological Association. Guidelines for nonsexist use of language. *American Psychologist*. 1975;30:682–684) and to be sensitive to the semantic description of persons with chronic diseases and disabilities, as outlined in *Medicine & Science in Sports & Exercise*® [Raven PR. Journal terminology: issues of sensitivity and accuracy. *Med. Sci. Sports Exerc*. 1991;23(11): 1217–8.]

As a general rule, only standardized abbreviations and symbols should be used. If unfamiliar abbreviations are employed, they should be defined when they first appear in the text. Authors should follow *Webster's Third New International Dictionary* for spelling, compounding, and division of words. Trademark names should be capitalized and the spelling verified. Chemical or generic names should precede the trade name or abbreviation of a drug the first time it is used in the text.

**Electronic Submission**

Manuscripts shall be submitted electronically via Editorial Manager® (www.editorialmanager.com/msse). Detailed information regarding registration and use of Editorial Manager® is found on the Web site. *Hard-copy manuscript submissions will not be accepted by the Editorial Office and will be discarded.*

Authors submitting manuscripts for review shall also submit:

- Completed mandatory submission form
- Manuscript submission fee (Non–ACSM member corresponding authors only)
- Letter of permission to reprint figures or tables (if applicable)

**Manuscript Requirements—Original Submissions**

The manuscript file must be in a document format, not PDF format. The manuscript shall be formatted so that it is set in Times Roman font with 12-point font size and has margins of 1" (all sides). Manuscript pages must be double-spaced with continuous line numbers. Typical manuscript length is approximately

20 pages including references, but excluding tables and figures.

Submit all figure and table files separately from the manuscript text file. Figures and tables are limited to six (6) total (e.g., 2 figures, 4 tables; 0 figures, 6 tables). *For original submissions and first review*, figures may be .tif, .eps, .jpeg, .gif, .doc(x), .ppt, or .pdf format.

**Revised Manuscripts**

Authors submitting revised manuscripts shall adhere to the above requirements and submit through Editorial Manager® (www.editorialmanager.com/msse). When submitting a revised manuscript, author point-by-point responses to reviewer comments must be a separate document. Figures should be resubmitted in industry-standard .tif or .eps format as these are the formats required for publication. Artwork requiring increased image resolution or failing the quality control check or will be returned to the author for correction.

In addition to these requirements, author-completed copyright transfer agreements must accompany revised manuscripts. Each author must complete and submit the journal's copyright transfer agreement, which includes a section on the disclosure of potential conflicts of interest based on the recommendations of the International Committee of Medical Journal Editors, "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" (www.icmje.org/update.html). The form is readily available on the manuscript submission page (http://www.editorialmanager.com/msse/) and can be completed and submitted electronically. Please note that authors may sign the copyright transfer agreement form electronically. For additional information about electronically signing this form, go to http://links.lww.com/ZUAT/A106.

**Order of Manuscript**

An original investigation should contain the following items and satisfy the given specifications.

- Title Page
    1. Title of no more than 85 characters, including spaces.
    2. Full names of the authors—Only those investigators who contributed substantially or who had a primary role in the research represented in the manuscript should be listed as authors. The Editor-in-Chief reserves the right to request that the author list be reduced.
    3. Institutional affiliation of each author clearly identified; linked to each author by use of superscript numbers
    4. Corresponding author name, mailing address, telephone, fax, and e-mail information
- Abstract
    1. Limit of 275 words, including numbers, abbreviations, and symbols
    2. Structure states purpose, methods, results, and conclusion
    3. Reference citations are not permitted
- Key Words
    1. Four (4) to six (6) words following the abstract
    2. Should not repeat terms or phrases from the title
- Introduction
    1. State clearly the purpose and hypothesis of the study
    2. Provide relevant references
    3. Do not exhaustively review the subject
- Methods
    1. Present subject information
    2. Describe the experimental subjects and their controls
    3. Insert “written informed consent” statement or animal-use statement and ethics committee approval statement (required) (see “Human & Animal Experimentation Policy Statements”)
    4. Identify the methods, apparatus, and procedures employed with sufficient details to allow others to reproduce the results
    5. Provide references for established methods and statistical procedures
    6. Provide rationale for use and include a description of possible limitations for utilized methods not well known

7. Denote statistical significance when appropriate and include detailed statistical analyses, mathematical derivation, or computer programs as supplemental digital content (SDC).

- Results
    1. Present findings of the study in the text, tables, or figures
    2. Do not include the same data in tables and figures
- Discussion
    1. Emphasize the original and important features of the study and avoid repeating all the data presented within the results section
    2. Incorporate the significance of the findings and the relationship(s) and relevance to published observations
    3. Provide only those conclusions that are supported by the study
- Acknowledgments
    1. Identify funding sources. Authors are required to state in the acknowledgments all funding sources, and the names of companies, manufacturers, or outside organizations providing technical or equipment support.
    2. Give credit to others who contributed to the development and results of the study.
- Conflict of Interest

    In particular, authors should:

    1. Disclose professional relationships with companies or manufacturers who will benefit from the results of the present study
    2. State that the results of the present study do not constitute endorsement by ACSM
- References

The reference list shall be in alphabetic order (rather than in the order of citation) and numbered. There shall not be more than 40 references for original investigations. Review articles are limited to 75 references. All references shall appear in the text. The format for references is that which has been adopted by the United States National Library of Medicine [Patrias K. *National Library of Medicine Recommended Formats for Bibliographic Citation*. Bethesda (MD): The

Library; 1991. Available from: NTIS, Springfield, VA; PB91-182030.] and employed in *Index Medicus*. For those not included in *Index Medicus*, adhere to the form established by the American National Standard for Bibliographic References. Examples of the types of references are as follows:

1. **Book**
    - Cohen J. *Statistical Power Analysis for the Behavioral Sciences*. 2nd ed. Hillsdale (NJ): Lawrence Erlbaum Associates; 1988. 567 p.
    - Paffenbarger RS, Hyde RT, Wing AL. Physical activity and physical fitness as determinants of health and longevity. In: Bouchard C, Shephard RJ, Stephens T, Sutton JR, McPherson BD, editors. *Exercise, Fitness, and Health*. Champaign: Human Kinetics; 1990. p. 33–48.
2. **Conference Proceedings**—Matthie JR, Withers PO, Van Loan MD, Mayclin PL. Development of a commercial complex bio-impedance spectroscopic (CBIS) system for determining intracellular water (ICW) and extracellular water (ECW) volumes. In: *Proceedings of the 8th International Conference on Electrical Bio-impedance*; 1992 Jul 28-31: Kuopio (Finland). University of Kuopio; 1992. p. 203–5.
3. **Doctoral Dissertation**—Crandall C. Alterations in human baroreceptor reflex regulation of blood pressure following 15 days of simulated microgravity exposure [dissertation]. Fort Worth (TX): University of North Texas; 1993. 100 p.
4. **Government Report**—U.S. Department of Health and Human Services. *Bone Health and Osteoporosis: A Report of the Surgeon General*. Rockville, MD: U.S. Department of Health and Human Services, Office of the Surgeon General; 2004. 436 p. Available from: U.S. GPO, Washington.
5. **Journal Article**—Blair SN, Ellsworth NM, Haskell WL, Stern MP, Farguhar JW, Wood PD. Comparison of nutrient intake in middle-aged men and women runners and controls. *Med Sci Sports Exerc*. 1981;13(5):310–5.
6. **E-Journal Article**—Vickers AJ. Time course of muscle soreness following different types of exercise. *BMC Musculoskeletal Disorders* [Internet]. 2001 [cited 2001 May 31];2(5). Available

from: http://www.biomedcentral.com/1471-2474/2/5. doi:10.1186/1471-2474-2-5.

7. **Web site home page**—American Heart Association Web site [Internet]. Dallas (TX): American Heart Association; [cited 2006 Jan 1]. Available from:http://www.americanheart.org.
8. **Abstract**—An abstract can be cited when it is the only source of information.

**Note:** In-text reference citations shall be baseline in parentheses, not superscripts [e.g., (14,15), not [14,15]]. Personal Internet Web sites, Master of Science theses, personal communications, or other unpublished material are not acceptable as references. All book references require page numbers. Journal abbreviations should follow the abbreviations of *Index Medicus* published by the Library of Congress. Use of et al.—If fewer than seven (7) authors are listed, all should be mentioned. When seven or more authors are named, list only the first three.

- Appendices

  Appendices are considered supplemental material and will not be published in the print journal. Appendices will appear online only. Submitted appendices shall meet the requirements given in the section "Supplemental Digital Content (SDC)."
- Figure Captions
  - Provide a caption for each figure
  - List captions together following references section

## 3. Technical Guidelines

### Terminology and Units of Measurement

To promote consistency and clarity of communication, authors should use standard terms generally acceptable to the field of exercise science and sports medicine.

The units of measurement shall be Système International d'Unités (SI). Permitted exceptions to SI are heart rate—beats per minute (bpm); blood pressure—mm Hg; gas pressure—mm Hg. When expressing compound units of measurement,

authors must locate the raised dot midway between lines to avoid confusion with periods; for example, $mL \cdot min^{-1} \cdot kg^{-1}$.

The basic and derived units most commonly used in reporting research in this journal include the following: mass—gram (g) or kilogram (kg); force—newton (N); distance—meter (m), kilometer (km); temperature—degree Celsius (°C); energy, heat, work—joule (J) or kilojoule (kJ); power—watt (W); torque—newton-meter (N·m); frequency—hertz (Hz); pressure—pascal (Pa); time—second (s), minute (min), hour (h); volume—liter (L), milliliter (mL); and amount of a particular substance—mole (mol), millimole (mmol). Selected conversion factors: 1 N = 0.102 kg (force); 1 J = 1 N·m = 0.000239 kcal = 0.102 kg·m; 1 kJ = 1000 N·m = 0.239 kcal = 102 kg·m; 1 W = 1 $J \cdot s^{-1}$ = 6.118 $kg \cdot m \cdot min^{-1}$.

**Sample Size**

Authors should justify the adequacy of their sample size by providing calculations regarding the power of their statistical tests. While there are different approaches that authors may take in performing these calculations, the book by Cohen is recommended as an appropriate starting point [Cohen J.*Statistical Power Analysis for the Behavioral Sciences*. 2nd ed. Hillsdale (NJ): Lawrence Erlbaum Associates; 1988. 567 p.].

**Formulas and Equations**

Simple in-text formulas and equations should be presented in a single line: $M = (a + b)/(x + y)$. More complex equations should be set displayed, and, if referenced in text, shall have an equation number:

$$\dot{V}O_{2(t)} = A_1(1 - e^{-(t-\delta_1)/\tau_1}) + A_2(1 - e^{-(t-\delta_2)/\tau_2}) \quad [1]$$

All unusual characters must be accompanied by a definition or explanation.

## Figures

*Medicine & Science in Sports & Exercise*® accepts electronic file artwork only. Captions are required for all figures and shall appear on a separate page at the end of the manuscript.

Guidelines (http://edmgr.ovid.com/lww-final/accounts/5StepsforArt.pdf):

- Each figure should be saved as a separate file without captions. Any figure with multiple parts should be sent as one file with each part labeled the way it is to appear in print.
- Files should be saved as and submitted in .tif or .eps format—jpeg, .gif, or files downloaded from the Internet *are not* acceptable due to low resolution.
- Black-and-white line art should be saved at 900–1200 dpi (dots per inch) resolution with monochrome, 1-bit color mode.
- Photographs, CT scans, radiographs, etc. should be saved at a resolution of at least 300 dpi.
- Combination photo–line art and grayscale images should be saved at 600–900 dpi.
- Color images should be scanned in CMYK (cyan, magenta, yellow, black) mode. Do not submit any figures in RGB (red, green, blue) mode.
- Lettering (symbols, letters, and numbers) should be between 8 and 12 points, with consistent spacing and alignment. Font face maybe serif (Times Roman) or sans serif (Arial).
- Line width should be ¾ point or greater.
- Any extra white or black space surrounding the image should be cropped. Ensure that subject-identifying information (i.e., faces, names, or any other identifying features) is cropped or opaqued.
- Artwork should be submitted in final size and should be cropped and rotated as it will appear in the final printed piece.

## Tables

- Tables should be double-spaced and designed to fit a one-column width (3¼ inches) or a two-column width (7 inches) on a single page. Large, multipage tables are candidates for supplemental digital content (SDC).
- Each table shall have a brief caption; explanatory matter should be in footnotes below the table.
- The table shall contain means and the units of variation (SD, SE, etc.) and must be free of nonsignificant decimal places.
- Abbreviations used in tables must be consistent with those used in the text and figures. Definition symbols should be listed in the order of appearance, determined by reading horizontally across the table and should be identified by standard symbols.

## Supplemental Digital Content (SDC)

Authors may submit supplemental digital content (SDC) that enhances their article's text via Editorial Manager. SDC may include standard media such as text documents, graphics, audio, video, etc. On the Attach Files page of the submission process, please select Supplemental Audio, Video, or Data for your uploaded file as the Submission Item. If an article with SDC is accepted, production staff will create a URL with the SDC file. The URL will be placed in the call-out within the article. SDC files are not copy-edited by LWW staff; they will be presented digitally as submitted. SDC content will appear online only and will not appear in print. For a list of all available file types and detailed instructions, please visit http://links.lww.com/A142. *Please note that SDC should not include cover letters to the editor, forms required by the editorial office, or items required in the manuscript file.*

### *SDC Callouts*

Supplemental digital content must be cited consecutively in the text of the submitted manuscript. Citations should include the type of material submitted (Audio, Figure, Table, etc.), be clearly labeled as "Supplemental Digital Content," include the sequential list number, and provide a description of the supplemental

content. All descriptive text should be included in the call-out as it will not appear elsewhere in the article.

*Example:* We performed many tests on the degrees of flexibility in the elbow (see Video, Supplemental Digital Content 1, which demonstrates elbow flexibility) and found our results inconclusive.

***List of Supplemental Digital Content***

A listing of Supplemental Digital Content must be submitted at the end of the manuscript file. Include the SDC number and file type of the Supplemental Digital Content. This text will be removed by our production staff and not be published.

*Example:* Supplemental Digital Content 1. wmv

***SDC File Requirements***

All acceptable file types are permissible up to 10 MB. For audio or video files greater than 10 MB, authors should first query the journal office for approval. For a list of all available file types and detailed instructions, please visit http://links.lww.com/A142.

**4. Human & Animal Experimentation Policy Statements**

*Failure to comply with the guidelines that follow and to guarantee such conformance by a statement in the manuscript will result in rejection of the manuscript.*

Studies and case reports involving the use of human subjects shall conform to the policy statement regarding the use of human subjects and written informed consent as published by *Medicine & Science in Sports & Exercise*®. All studies involving animal experimentation shall be conducted in conformance with the policy statement of the American College of Sports Medicine on research with experimental animals as published by *Medicine & Science in Sports & Exercise*®.

**Policy Statement Regarding the Use of Human Subjects and Informed Consent**

By law, any experimental subject or clinical patient who is exposed to possible physical, psychological, or social injury must give informed consent prior to participating in a proposed project. Informed consent can be defined as the knowing consent of an individual or his legally authorized representative so situated as to be able to exercise free power of choice without undue inducement or any element of force, fraud, deceit, duress, or other form of constraint or coercion.

The Editorial Board of *Medicine & Science in Sports & Exercise*® requires that all appropriate steps be taken in obtaining the informed consent of any and all human subjects employed by investigators submitting manuscripts for review and possible publication. In most cases, written informed consent should be obtained by having the subject read a document (an Informed Consent Form) presenting all information pertinent to the investigation or project and affixing a signature indicating that the document has been read and consent given to participation under the conditions described therein. In some cases, usually when risks to subjects are very low (e.g., survey research), the Institutional Review Board of record may approve the conduct of the investigation and declare the study to be exempt from the usual requirement of obtaining written informed consent, in lieu of obtaining the participants' verbal consent to participate. Information presented at the time of consent should be provided in a way that it is easily understood by the subjects and provided in a language in which the subjects are fluent.

Investigators are requested to consider the following items for inclusion in an Informed Consent Form, or process, as appropriate to the particular project:

- A general statement of the background of the project and the project objectives.
- A fair explanation of the procedures to be followed and their purposes, identification of any procedures that are experimental, and description of any and all risks attendant to the procedures.

- A description of any benefits to be reasonably expected and, in the case of treatment, disclosure of any appropriate alternative procedures that might be advantageous to the subject.
- An offer to answer any queries of the subject concerning procedures or other aspects of the project.
- An instruction that the subject is free to withdraw consent and to discontinue participation in the project or activity at any time without prejudice to the subject.
- An instruction that, in the case of questionnaires and interviews, the subject is free to deny answer to specific items or questions.
- An instruction that, if services or treatment are involved in the setting or context of the project, they will be neither enhanced nor diminished as a result of the subject's decision to volunteer or not to volunteer participation in the project.
- An explanation of the procedures to be taken to ensure the confidentiality of the data and information to be derived from the subject. If subjects are to be identified by name in the manuscript, permission for same should be obtained in the Informed Consent Form or obtained in writing at a later date.

If the subject is to be videotaped or photographed in any manner, this must be disclosed in the Informed Consent Form. The subject must be advised as to who will have custody of such videotapes or photographs, who will have access to the tapes or photographs, how the tapes or photographs are to be used, and what will be done with them when the study is completed.

The informed consent document, or process, shall not contain any exculpatory language or any other waiver of legal rights releasing, or appearing to release, an investigator, project director, or institution from liability. If a consent form is used, at the bottom of the form, provision shall be made for the signature of the subject (and/or a legally authorized representative) and the date. It is generally advisable to precede this with a statement to the effect that the subject and/or representative have read the statement and understand it. In the case of minors, one or both parents should sign (as appropriate). For minors of sufficient maturity, signatures should be obtained from the subject and the parent(s).

The Editorial Board endorses the Declaration of Helsinki of the World Medical Association as regards the conduct of clinical research. Physicians are expected to comply with the principles set forth in this declaration when research involves the use of patients. In the case of psychological research, investigators will be expected to comply with the principles established by the American Psychological Association. (American Psychological Association.*Ethical Principles in the Conduct of Research with Human Participants.* Washington, DC: American Psychological Association; 1982.). The use of subjects should be approved by an ethics committee prior to the investigation and shall be stated in the Methods section of the submitted manuscript.

It will not be necessary for an author to describe in the manuscript the specific steps that were taken to obtain informed consent, to ensure confidentiality of results, or to protect the privacy rights of participating subjects. It will be satisfactory for the author to indicate that, “informed consent was obtained from the subject,” or by similar wording. Manuscripts reporting research approved for conduct as exempt from the requirement for obtaining written informed consent should identify the specific Institutional Review Board of record that made that determination. It will be understood by the editors that such a statement indicates the author’s guarantee of compliance with the directives presented above.

**Policy Statement of the American College of Sports Medicine on Research with Experimental Animals**

The ability of science to enhance the well being of humans and animals depends directly on advancements made possible by research, much of which requires the use and availability of experimental animals. Therefore, all who propose to use animals for research, education, or testing purposes must assume the responsibility for their general welfare. It is essential to recognize and to appreciate that the intent of scientific research is to provide results that will advance knowledge for the general and specific benefits of humans and animals. To accomplish these goals, the American College of Sports Medicine (ACSM) will support research of high scientific merit that includes the use of experimental animals.

Before the College will consider supporting research projects, the College must receive written assurances from the institution that the policies and procedures detailed by the Institute for Laboratory Animal Research (Institute for Laboratory Animal Research. *Guide for the Care and Use of Laboratory Animals*. Washington, DC: National Academy Press; 1996.) and proclaimed in the Animal Welfare Act (PL89-544, PL91-979, and PL94-279) are policies of the institution. Furthermore, ACSM endorses the rules, procedures, and recommendations for the care of laboratory animals as advocated by the American Association for Accreditation of Laboratory Animal Care (AAALAC). Support for research and publication of research findings by ACSM require that the institution where the research was conducted confirm it has filed a National Institutes of Health assurance and/or has AAALAC approved facilities.