Journal of Pain Research

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CASE SERIES

Markers of Tissue Deterioration and Pain on Earth and in Space

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Purpose: Pain is an understudied physiological effect of spaceflight. Changes in inflammatory and tissue degradation markers are often associated with painful conditions. Our aim was to evaluate the changes in markers associated with tissue deterioration after a short-term spaceflight.

Patients and Methods: Plasma levels of markers for systemic inflammation and tissue degeneration markers were assessed in two astronauts before and within 24 h after the 17-day Axiom Space AX-1 mission.

Results: After the spaceflight, C-reactive protein (CRP) was reduced in both astronauts, while INFγ, GM-CSF, TNFα, BDNF, and all measured interleukins were consistently increased. Chemokines demonstrated variable changes, with consistent positive changes in CCL3, 4, 8, 22 and CXCL8, 9, 10, and consistent negative change in CCL8. Markers associated with tissue degradation and bone turnover demonstrated consistent increases in MMP1, MMP13, NTX and OPG, and consistent decreases in MMP3 and MMP9.

Conclusion: Spaceflight induced changes in the markers of systemic inflammation, tissue deterioration, and bone resorption in two astronauts after a short, 17-day, which were often consistent with those observed in painful conditions on Earth. However, some differences, such as a consistent decrease in CRP, were noted. All records for the effect of space travel on human health are critical for improving our understanding of the effect of this unique environment on humans.

Keywords: spaceflight, astronaut, cytokine, interleukin, chemokine, bone turnover

Introduction

Pain is an understudied physiological response to spaceflight, even though it is commonly reported by astronauts during and after missions to space.[1] It is well known that musculoskeletal tissues need loading to maintain a good physiology,[2] and degradation of bone and cartilage are associated with painful conditions, such as osteoarthritis, low back pain, and osteolytic bone metastasis.[3] Microgravity decreases physical demand on the body, resulting in a decrease in muscle mass[4] and alterations in the intervertebral discs, leading to back and neck pain,[5,6] bone loss,[7] and other symptoms like immune and bone turnover dysfunction in the elderly.[8,9] In general, changes in the musculoskeletal system occur relatively slowly, making it difficult to study the effects of short-duration flights. Nevertheless, inflammatory markers associated with tissue deterioration often increase early in the process, and bone resorption markers specifically were shown to significantly increase within 10 to 14 days of spaceflight,[7] suggesting their potential usefulness for assessing the initiation of the degradation processes in musculoskeletal system during short-duration spaceflight. Importantly, interleukins (IL), in

Received: 16 November 2023
Accepted: 25 April 2024
Published: 8 May 2024

particularly IL-6,[10] chemokines, cytokines that regulate osteoclast function, tumor necrosis factor (TNF) α, receptor activator of nuclear factor κB ligand (RANKL) and osteoprotegerin (OPG),[11] as well as other inflammatory markers,[12] have been shown to be consistently associated with pain in clinical research.

The aim of this study was to evaluate the changes of systemic inflammation markers associated with tissue deterioration in general and bone resorption specifically after exposure to microgravity for a 17-day-long spaceflight in astronauts on the first all-private Axiom Space AX-1 mission to the International Space Station. This study is complementary to the recent report that, for the first time, investigated the somatosensory changes in the Axiom AX-1 astronauts.[1]

Materials and Methods

Population and Consent

This study was approved by the Institutional Review Board (IRB) of the McGill University Health Center (MUHC: 2022–7768), the IRB of the National Aeronautics and Space Administration (NASA STUDY 00000403) by the University of Texas - M.D. Anderson (Reliance Agreement M.D. Anderson - NASA 2021–1179). All study procedures were conducted according to the guidelines of the 2013 Declaration of Helsinki. Two crew members of Axiom Space's AX-1, launched on April 8, 2022, at 11:17 AM ET and splashed down on April 25, 2022, at 1:06 PM ET agreed to participate. The informed consent briefing was in October 2021, informed consent forms were signed on December 2021, the revised informed consent forms (an increase in the total blood collection from 30 mL to 37 mL at each time point) were signed in March 2022. The crew members remained in orbit for seventeen days and completed 240 orbits. Validated pain questionnaires, qualitative interviews and quantitative sensory testing were performed before, during and after the space mission and reported in Sauer et al.[1]

Specimen Collection and Processing

Written consent was obtained from two male astronauts to collect peripheral venous whole blood samples using BD P100 blood collection tubes within two weeks of departure, and 24 h after landing, centrifuged at 1300 G, room temperature for 10 min. Plasma was aliquoted into dedicated cryotubes and stored at −80°C until further processing.

Specimen Analysis

Thirty-five analytes were selected for multiplex protein analysis. The analysis was performed according to the manufacturer's instructions (ThermoFisher) using three custom kits: PPX-24-MX9HKUR containing brain derived neurotrophic factor (BDNF), epithelial-derived neutrophil-activating peptide 7 (CXCL5), myeloid progenitor inhibitory factor 2 (CCL24), granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon (IFN) gamma, hematopoietin 1 (IL-1 alpha), lymphocyte activating factor (IL-1 beta), IL-2, IL-4, IL-6, IL-8, IL-10, IL-17A, IL-21, IFN gamma-induced protein 10 (IP-10/CXCL10), monocyte chemoattractant protein (MCP) −3, macrophage derived chemokine (MDC/CCL22), monokine induced by gamma IFN (CXCL9), macrophage inflammatory protein 1-alpha (CCL3), macrophage inflammatory protein 1-beta (CCL4), matrix metalloproteinase (MMP) 1, MMP-13, and TNFα; granulocyte chemotactic protein-2 (CXCL6), MCP-1, TNF superfamily member 14 (LIGHT), beta nerve growth factor (NGF-beta) and MCP-2; and CRP, MMP-3, transforming growth factor (TGF) beta, MMP-9, Osteopontin and regulated on activation, normal T cell expressed and secreted (RANTES/CCL5). Protein concentrations in pg/mL were acquired from a MAGPIX (Luminex) using 30 ul of plasma. Four analytes (LIGHT, NGF-beta, TGF-beta and GRO-alpha) were below the detection threshold and were therefore omitted from the analysis. Median fluorescence intensity (MFI) was measured, and the concentration from each analyte was calculated using a 7-point standard curve by the ThermoFisher ProcartaPlex analysis platform. ELISA kits were used for the following analytes: human cross linked N-telopeptide of type I collagen (NTX, Cedarlane MBS705111); human procollagen I C-terminal (PICP, Cedarlane MBS2702057), human osteoprotegerin (OPG, ABclonal Science Inc RK00317) human TRANCE/TNFSF11/RANKL (ABclonal Science Inc, RK00341), and human osteocalcin (ABclonal Science Inc, RK09258).

Rapid Literature Review Methodology

Rapid reviews were performed on October 10, 2023, in Medline (Ovid) using the search strategies that combined a) the list of biomarkers measured in the study, and b) pain as a MESH term and keyword. Resulting studies were screened by a single reviewer and were included in the analysis if they were performed with human subjects, measured biomarkers of interest in plasma or serum, and reported association with pain outcomes. From each manuscript, we extracted data for publication first author and date, disease studied and total number of study subjects, pain measurement method, reported biomarkers and the direction of association between pain and each biomarker (positive, negative, or undetermined (variable or no association)). The diseases were combined as follows: abdominal pain (AP): appendicitis, abdominal pain and kidney pain; back pain (BP): chronic low back pain, spinal tuberculosis, and lumbar disc herniations; chronic malignant pain (CMP): cancer: chronic non-malignant pain (CNMP): complex regional pain syndrome, chronic widespread pain, depression, fibromyalgia, and neuropathic pain; chest pain (CP): myocardial infarction and coronary syndromes; head and neck pain (HNP): temporomandibular disorder and migraines; musculoskeletal pain (MSP): foot pain, growing pain, shoulder pain, and non-arthritis joint pain; osteoarthritis (OA); and pelvic pain (PP): chronic pelvic pain syndrome, endometriosis, labor pain, and prostatitis.

Data Analysis and Presentation

Raw data are presented in tables; for figures, percentage difference from pre-flight values was calculated and $d = \left(x_{postflight} - x_{preflight}\right) * 100/x_{preflight}$. Figures for disease-marker association were developed using Matlab using the following rules: the word's size is proportional to the number of included studies that report it; each connecting line represents an article, and the width of the line is proportional to the study size. To assign the association of specific biomarkers with pain, each study was given a value of 1 for a positive association, 0 for no association, and −1 for a negative association. For each biomarker, the sum of 2 or greater was interpreted as a general positive association with pain (red color), the sum of −2 or less as a general negative association (blue color), and a sum of −1, 0 or 1 as no association (grey color). No statistical analysis was performed for the experimental values obtained from the two astronauts.

Results and Discussion

The participants were healthy male astronauts 53 and 64 years of age with no history of chronic pain, spinal surgery, or peripheral nervous system disorder. After the 17-day long spaceflight, CRP was reduced in both astronauts, while INFγ, GM-CSF, TNFα, BDNF, and all measured interleukins were consistently increased (Tables 1 and 2, Figure 1A). To examine the reported association of these markers with painful conditions, we performed a rapid review, which retrieved 362 studies, of which 42 were included in the final analysis.[13–54] TNFα, IL-6, and CRP were reported to be positively associated with pain, specifically with CNMP, PP, CP, and OA (Figure 1B). Of interest, even though TNFα and IL6 were consistently increased in astronauts after the spaceflight, CRP was consistently decreased. Thus, with the notable exception of CRP, spaceflight-associated changes in cytokines were like those observed in painful conditions.

Table 1 Spaceflight-Induced Changes in Cytokines and Growth Factors. Preflight and Postflight Values in Pg/Ml are Given for Astronauts A1 and A2

	CRP	INFγ	GM-CSF	TNFα	BDNF
A1 pre	1369.58	29.68	107.51	8.86	60.96
A1 post	813.95	150.44	276.22	45.80	80.32
A2 pre	1695.19	33.25	98.08	10.17	20.15
A2 post	920.07	131.26	216.73	37.33	36.76

Table 2 Spaceflight-Induced Changes in Interleukins. Preflight and Postflight Values in Pg/Ml are Given for Astronauts A1 and A2

	IL1a	IL1b	IL2	IL4	IL6	IL8	IL10	IL17a	IL21
A1 pre	4.38	9.87	36.69	16.57	53.48	5.58	7.37	35.56	245.09
A1 post	7.51	49.92	89.56	66.33	121.88	10.25	27.14	141.05	349.82
A2 pre	1.10	16.40	27.83	16.82	51.48	1.50	8.73	39.69	ND
A2 post	3.69	50.85	68.75	54.81	119.12	5.74	22.45	116.57	58.84

Chemokines demonstrated variable changes, with consistent positive changes in CCL3, 4, 8, 22 and CXCL8, 9, 10, consistent negative change in CCL8, and variable or no changes in CCL5, 24, and CXCL5, 6 (Tables 3 and 4, Figure 2A). To examine reported association of these markers with painful conditions, we performed a rapid review which retrieved 211 studies, of which 17 were included in the final analysis.[18,39,55–68] Several chemokines, including CCL3, 4, 5, and 24 and CXCL5 and 10, were reported to be negatively associated with pain, specifically with CNMP, PP, BP, and OA (Figure 2B). CXCL8, most reported for its association with painful conditions, was also reported to be both positively and negatively associated with pain outcomes. Thus, spaceflight-associated changes in chemokines are different from those observed in painful conditions.

Markers associated with tissue degradation and bone turnover were similarly variable, with consistent increases in MMP1, MMP13, NTX, and OPG, consistent decreases in MMP3 and MMP9, and variable changes in PICP, OPN and OCN (Tables 5 and 6, Figure 3A). To examine reported association of these markers with painful conditions, we performed a rapid review which retrieved 373 studies, of which 24 were included in the final analysis.[56,69–91] Bone

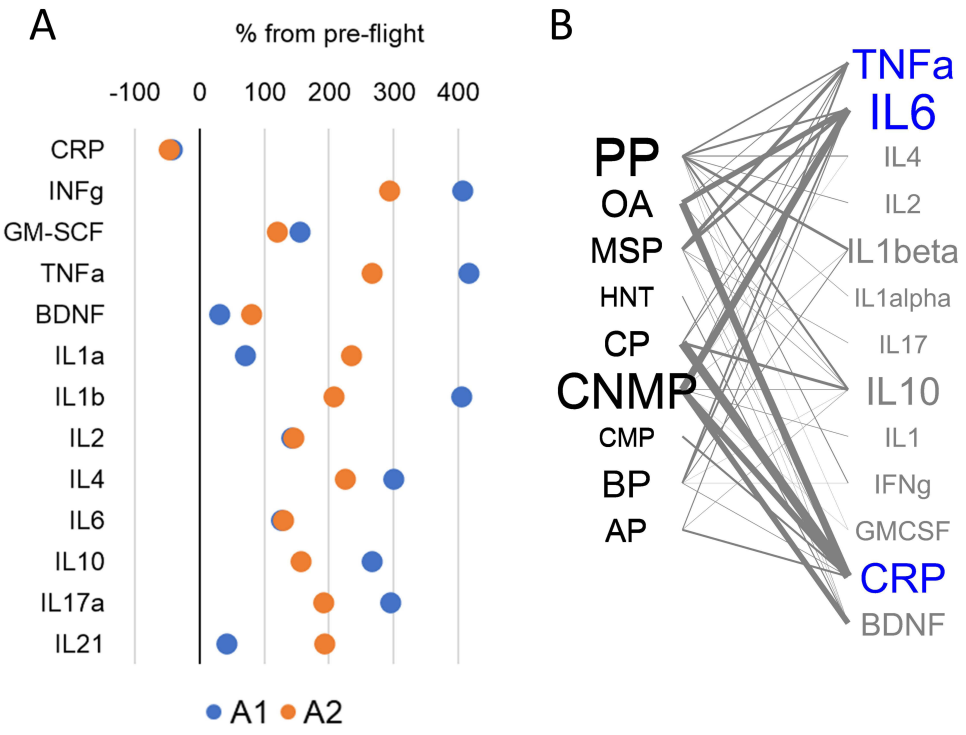

Figure 1 Spaceflight-induced changes in cytokines and growth factors. (**A**) For each astronaut (A1 and A2), the percentage change from pre-flight was calculated and plotted for reported cytokines and growth factors. (**B**) Map of associations between painful conditions and the reported cytokines and growth factors. The size of the letters is proportional to the frequency of mention; the thickness of the connecting lines is proportional to the total number of patients in reported studies, bleu font indicates consistently positive association, grey variable association or no association.

Abbreviations: PP, pelvic pain; OA, osteoarthritis; MSP, musculoskeletal pain; HNT, head and neck pain; CP, chest pain; CNMP, chronic non-malignant pain; CMP, chronic malignant pain; BP, back pain; AP, abdominal pain.

Table 3 Spaceflight-Induced Changes in CCL Chemokines. Preflight and Postflight Values in Pg/Ml are Given for Astronauts A1 and A2

	CCL3	CCL4	CCL5	CCL7	CCL8	CCL22	CCL24
A1 pre	13.04	60.96	357.15	37.65	4.24	130.39	60.60
A1 post	18.32	80.32	178.94	69.62	2.51	229.86	64.04
A2 pre	1.98	20.15	289.24	47.16	6.07	143.47	62.00
A2 post	6.77	36.76	308.97	72.72	4.90	168.19	63.35

Table 4 Spaceflight-Induced Changes in CXCL Chemokines. Preflight and Postflight Values in Pg/Ml are Given for Astronauts A1 and A2

	CXCL5	CXCL6	CXCL9	CXCL10
A1 pre	122.39	7.51	28.51	27.84
A1 post	194.40	6.88	34.56	36.75
A2 pre	279.20	14.30	1.98	30.96
A2 post	242.47	11.21	6.77	65.34

resorption markers (BRM) and bone formation markers (BFM) were positively associated with pain in OA, MSP, and BP, and MMP9 was positively associated with CP and PP (Figure 3B). Thus, the increase in bone resorption marker NTX in astronauts is similar to the commonly reported increase in BRM in painful musculoskeletal conditions, however MMP9 was consistently decreased in astronauts and bone formation marker, PINP was variably affected.

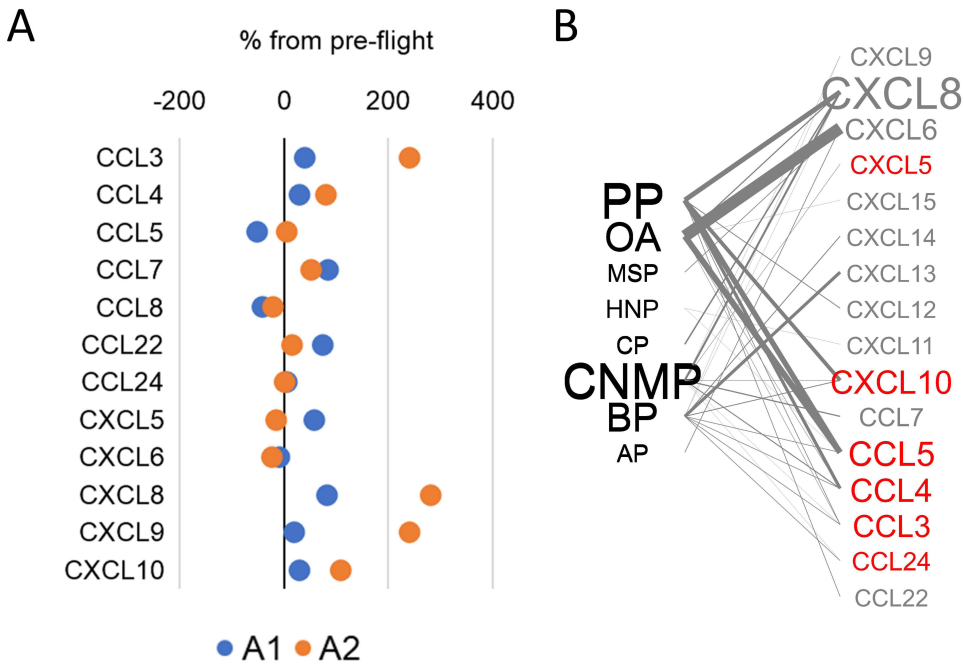

Figure 2 Spaceflight-induced changes in chemokines. (**A**) For each astronaut (A1 and A2), the percentage change from pre-flight was calculated and plotted for reported chemokines. (**B**) Map of associations between painful conditions and the reported chemokines. The size of the letters is proportional to the frequency of mention; the thickness of the connecting lines is proportional to the total number of patients in reported studies, red font indicates consistently negative association, grey font: variable association or no association.

Abbreviations: PP, pelvic pain; OA, osteoarthritis; MSP, musculoskeletal pain; HNT, head and neck pain; CP, chest pain; CNMP, chronic non-malignant pain; BP, back pain; AP, abdominal pain.

Table 5 Spaceflight-Induced Changes in MMPs. Preflight and Postflight Values in Pg/Ml are Given for Astronauts A1 and A2

	MMP1	MMP3	MMP9	MMP13
A1 pre	9.16	642.40	677.05	116.27
A1 post	42.65	454.07	506.42	281.24
A2 pre	13.64	760.83	449.93	133.96
A2 post	36.61	320.70	307.30	246.02

Table 6 Spaceflight-Induced Changes in Bone-Related Factors. Preflight and Postflight Values in Pg/Ml are Given for Astronauts A1 and A2

	NTX	PICP	OPN	OCN	OPG
A1 pre	ND	185.20	6072.36	3.73	2.64
A1 post	26.00	284.67	6857.75	4.76	4.10
A2 pre	ND	195.40	10,810.12	4.69	3.11
A2 post	14.59	130.53	4551.06	4.95	4.42

Limitations

Astronauts are exposed to multiple stressors, including microgravity, noise and vibrations, sleep disruptions, and confinement.[92] Within the scope of the study, it is impossible to attribute the observed changes to any of the specific stressors. This case study provides data for two astronauts only, making it impossible to reach concrete conclusions.

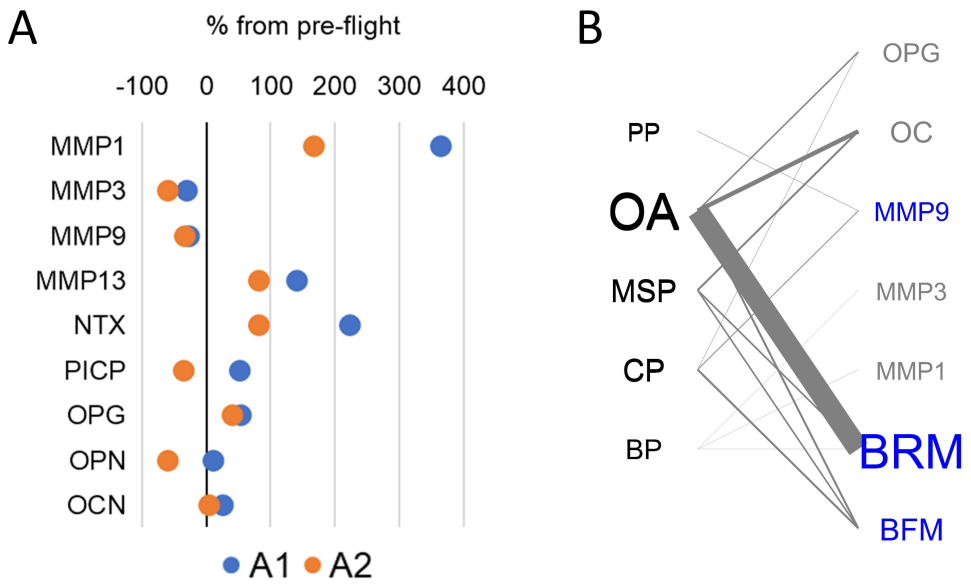

Figure 3 Spaceflight-induced changes in tissue degradation and bone turnover factors. (**A**) For each astronaut (A1 and A2), the percentage change from pre-flight was calculated and plotted for reported factors. (**B**) Map of associations between painful conditions and the reported factors. The size of the letters is proportional to the frequency of mention; the thickness of the connecting lines is proportional to the total number of patients in reported studies, blue font indicates consistently positive association, red font indicates consistently negative association, grey font: variable association or no association.

Abbreviations: PP, pelvic pain; OA, osteoarthritis; MSP, musculoskeletal pain; HNT, head and neck pain; CP, chest pain; CNMP, chronic non-malignant pain; CMP, chronic malignant pain; BP, back pain; AP, abdominal pain.

However, our studies demonstrate for the first time the changes in the somatosensory system[1] and biomarkers associated with pain (current study), paving the way for future studies that can be combined to improve our understanding of the effects of spaceflight on pain. Another limitation is related to the lack of blood samples taken in flight due to difficulties in obtaining samples as well as in finding time in the busy schedule of astronauts in space missions.

Conclusion

This study reports the changes in the markers of systemic inflammation, tissue deterioration, and bone resorption in two astronauts after a short, 17-day space travel. While some changes are consistent with those observed in painful conditions on Earth, we have noted some differences, such as a consistent decrease in CRP. Although no conclusions can be reached with two participants in the study, since space travel remains very costly and uncommon exposure to this unique environment, every record of the effect of space travel on human health is critical in improving our understanding of the effect of spaceflight on humans.

Abbreviations

AP, abdominal pain; BDNF, brain derived neurotrophic factor; BFM, bone formation markers; BP, back pain; BRM, bone resorption markers; CCL24, myeloid progenitor inhibitory factor 2; CCL3, macrophage inflammatory protein 1-alpha; CCL4, macrophage inflammatory protein 1-beta; CMP, chronic malignant pain; CNMP, chronic non-malignant pain; CP, chest pain; CRP, c-reactive protein; CRP, C-reactive protein; CXCL5, epithelial-derived neutrophil-activating peptide 7; CXCL6, granulocyte chemotactic protein-2; CXCL9, monokine induced by gamma IFN; GM-CSF, granulocyte-macrophage colony-stimulating factor; HNP, head and neck pain; IFN, interferon; IL-1 alpha, hematopoietin 1; IL-1 beta, lymphocyte activating factor; IL, interleukins; IP-10/CXCL10, IFN gamma-induced protein 10; IRB, Institutional Review Board; LIGHT, TNF superfamily member 14; MCP, monocyte chemoattractant protein; MDC/CCL22, macrophage-derived chemokine; MFI, Median fluorescence intensity; MMP, matrix metalloproteinase; MSP, musculoskeletal pain; NGF-beta, beta nerve growth factor; NTX, human cross linked N-telopeptide of type I collagen; OA, osteoarthritis; OPG, osteoprotegerin; PICP, human procollagen I C-terminal; PP, pelvic pain; RANKL, receptor activator of nuclear factor κB ligand; RANTES/CCL5, normal T cell expressed and secreted; TGF, transforming growth factor; TNF, tumor necrosis factor.

Acknowledgments

This work was supported by the Montreal Children's Hospital Foundation.

Disclosure

Dr Jean Ouellet reports grants from AO North America, outside the submitted work. The author reports no other conflicts of interest in this work.

References

1. Sauer AK, Vigouroux M, Dougherty PM, Cata JP, Ingelmo PM. Pain experience and sensory changes in astronauts during and after short-lasting commercial spaceflight: a proof-of-concept study. *J Pain Res*. 2023;16:4253–4266. doi:10.2147/jpr.S440630
2. Willie BM, Zimmermann EA, Vitienes I, Main RP, Komarova SV. Bone adaptation: safety factors and load predictability in shaping skeletal form. *Bone*. 2020;131:115114. doi:10.1016/j.bone.2019.115114
3. Puntillo F, Giglio M, Paladini A, et al. Pathophysiology of musculoskeletal pain: a narrative review. *Ther Adv Musculoskelet Dis*. 2021;13:1759720x21995067. doi:10.1177/1759720x21995067
4. Lee PHU, Chung M, Ren Z, Mair DB, Kim DH. Factors mediating spaceflight-induced skeletal muscle atrophy. *Am J Physiol Cell Physiol*. 2022;322(3):C567–C580. doi:10.1152/ajpcell.00203.2021
5. Chang DG, Healey RM, Snyder AJ, et al. Lumbar spine paraspinal muscle and intervertebral disc height changes in astronauts after long-duration spaceflight on the international space station. *Spine*. 2016;41(24):1917–1924. doi:10.1097/brs.0000000000001873
6. Burkhart K, Allaire B, Bouxsein ML. Negative effects of long-duration spaceflight on paraspinal muscle morphology. *Spine*. 2019;44(12):879–886. doi:10.1097/brs.0000000000002959
7. Stavnichuk M, Mikolajewicz N, Corlett T, Morris M, Komarova SV. A systematic review and meta-analysis of bone loss in space travelers. *NPJ Microgravity*. 2020;6:13. doi:10.1038/s41526-020-0103-2
8. Nagy EE, Nagy-Finna C, Popoviciu H, Kovács B. Soluble biomarkers of osteoporosis and osteoarthritis, from pathway mapping to clinical trials: an update. *Clin Interv Aging*. 2020;15:501–518. doi:10.2147/cia.S242288

9. Akbik OS, Ban VS, MacAllister MC, Aoun SG, Bagley CA. Genetic and serum markers in adult degenerative scoliosis: a literature review. *Spine Deform*. 2022;10(3):479–488. doi:10.1007/s43390-021-00451-y
10. Zhou YQ, Liu Z, Liu ZH, et al. Interleukin-6: an emerging regulator of pathological pain. *J Neuroinflammation*. 2016;13(1):141. doi:10.1186/s12974-016-0607-6
11. Kwan Tat S, Padrines M, Théoleyre S, Heymann D, Fortun Y. IL-6, RANKL, TNF-alpha/IL-1: interrelations in bone resorption pathophysiology. *Cytokine Growth Factor Rev*. 2004;15(1):49–60. doi:10.1016/j.cytogfr.2003.10.005
12. DeVon HA, Piano MR, Rosenfeld AG, Hoppensteadt DA. The association of pain with protein inflammatory biomarkers: a review of the literature. *Nurs Res*. 2014;63(1):51–62. doi:10.1097/nnr.0000000000000013
13. Al-Rawaf HA, Gabr SA, Alghadir AH. Vitamin D deficiency and molecular changes in circulating MicroRNAs in older adults with lower back pain. *Pain Res Manag*. 2021;6662651. doi:10.1155/2021/6662651
14. Albertoni Giraldes AL, Salomao R, Leal PD, Brunialti MK, Sakata RK. Effect of intravenous lidocaine combined with amitriptyline on pain intensity, clinical manifestations and the concentrations of IL-1, IL-6 and IL-8 in patients with fibromyalgia: a randomized double-blind study. *Int J Rheum Dis*. 2016;19(10):946–953. doi:10.1111/1756-185X.12904
15. Alghadir AH, Gabr SA, Rizk AA. Plasmatic adipocyte biomarkers and foot pain associated with flatfoot in schoolchildren with obesity. *Rev Assoc Med Bras*. 2019;65(8):1061–1066. doi:10.1590/1806-9282.65.8.1061
16. Alstergren P, Kopp S. Insufficient endogenous control of tumor necrosis factor-alpha contributes to temporomandibular joint pain and tissue destruction in rheumatoid arthritis. *J Rheumatol*. 2006;33(9):1734–1739.
17. Ang DC, Moore MN, Hilligoss J, Tabbey R. MCP-1 and IL-8 as pain biomarkers in fibromyalgia: a pilot study. *Pain Med*. 2011;12(8):1154–1161. doi:10.1111/j.1526-4637.2011.01179.x
18. Bäckryd E, Ghafouri B, Larsson B, Gerdle B. Plasma pro-inflammatory markers in chronic neuropathic pain: a multivariate, comparative, cross-sectional pilot study. *Scand J Pain*. 2016;10:1–5. doi:10.1016/j.sjpain.2015.06.006
19. Barisic I, Ljutic D, Vlak T, Bekavac J, Jankovic S. Laboratory and sonographic findings in dialyzed patients with bilateral chronic knee pain versus dialyzed asymptomatic patients. *Coll Antropol*. 2007;31(2):489–494.
20. Brennan ML, Penn MS, Van Lente F, et al. Prognostic value of myeloperoxidase in patients with chest pain. *N Engl J Med*. 2003;349(17):1595–1604.
21. Cavusoglu E, Ruwende C, Chopra V, et al. Adiponectin is an independent predictor of all-cause mortality, cardiac mortality, and myocardial infarction in patients presenting with chest pain. *Eur Heart J*. 2006;27(19):2300–2309.
22. de Queiroz BZ, Pereira DS, Lopes RA, et al. Association between the plasma levels of mediators of inflammation with pain and disability in the elderly with acute low back pain: data from the Back Complaints in the Elders (BACE)-Brazil Study. *Spine*. 2016;41(3):197–203. doi:10.1097/BRS.0000000000001214
23. Freidin MB, Wells HRR, Potter T, Livshits G, Menni C, Williams FMK. Metabolomic markers of fatigue: association between circulating metabolome and fatigue in women with chronic widespread pain. *Biochim*. 2018;1864(2):601–606. doi:10.1016/j.bbadis.2017.11.025
24. Guo H, Xu YM, Ye ZQ, Yu JH. [Levels of cytokines and heat-shock protein 70 in the seminal plasma of patients with chronic bacterial prostatitis and chronic prostatitis/chronic pelvic pain syndrome]. *Zhonghua Nan Ke Xue*. 2012;18(12):1088–1092. Chinese.
25. Jasim H. Topical review - salivary biomarkers in chronic muscle pain. *Scand J Pain*. 2023;23(1):3–13. doi:10.1515/sjpain-2022-0112
26. Jasim H, Carlsson A, Hedenberg-Magnusson B, Ghafouri B, Ernberg M. Saliva as a medium to detect and measure biomarkers related to pain. *Sci Rep*. 2018;8(1):3220. doi:10.1038/s41598-018-21131-4
27. Jasim H, Ghafouri B, Carlsson A, Hedenberg-Magnusson B, Ernberg M. Daytime changes of salivary biomarkers involved in pain. *J Oral Rehabil*. 2020;47(7):843–850. doi:10.1111/joor.12977
28. Jasim H, Ghafouri B, Gerdle B, Hedenberg-Magnusson B, Ernberg M. Altered levels of salivary and plasma pain related markers in temporomandibular disorders. *J Headache Pain*. 2020;21(1):105. doi:10.1186/s10194-020-01160-z
29. Kamieniak P, Bielewicz JM, Grochowski C, et al. IFN-gamma correlations with pain assessment, radiological findings, and clinical intercourse in patient after lumbar microdiscectomy: preliminary study. *Dis Markers*. 2020:1318930. doi:10.1155/2020/1318930
30. Khadra A, Fletcher P, Luzzi G, Shattock R, Hay P. Interleukin-8 levels in seminal plasma in chronic prostatitis/chronic pelvic pain syndrome and nonspecific urethritis. *BJU Int*. 2006;97(5):1043–1046.
31. Kinfe TM, Muhammad S, Link C, Roeske S, Chaudhry SR, Yearwood TL. Burst spinal cord stimulation increases peripheral antineuroinflammatory interleukin 10 levels in failed back surgery syndrome patients with predominant back pain. *Neuromodulation*. 2017;20(4):322–330. doi:10.1111/ner.12586
32. Kovacs D, Eszlari N, Petschner P, et al. Interleukin-6 promoter polymorphism interacts with pain and life stress influencing depression phenotypes. *J Neural Transm*. 2016;123(5):541–548. doi:10.1007/s00702-016-1506-9
33. Lee E, Nelson OL, Puyana C, et al. Association between C-reactive protein and radiotherapy-related pain in a tri-racial/ethnic population of breast cancer patients: a prospective cohort study. *Breast Cancer Res*. 2019;21(1):70. doi:10.1186/s13058-019-1151-y
34. Li D, Song X, Huang H, Huang H, Ye Z. Association of Parkinson's disease-related pain with plasma interleukin-1, interleukin-6, interleukin-10, and tumour necrosis factor-α. *Neurosci Lett*. 2018;683:181–184. doi:10.1016/j.neulet.2018.07.027
35. Liu YC, Hsiao HT, Wang JC, Wen TC, Chen SL. TGF-β1 in plasma and cerebrospinal fluid can be used as a biological indicator of chronic pain in patients with osteoarthritis. *PLoS One*. 2022;17(1):e0262074. doi:10.1371/journal.pone.0262074
36. McCann CJ, Glover BM, Menown IB, et al. Investigation of a multimarker approach to the initial assessment of patients with acute chest pain. *Adv Ther*. 2009;26(5):531–534. doi:10.1007/s12325-009-0032-7
37. Miller LJ, Fischer KA, Goralnick SJ, et al. Nerve growth factor and chronic prostatitis/chronic pelvic pain syndrome. *Urology*. 2002;59(4):603–608. doi:10.1016/s0090-4295(01)01597-7
38. Nieman DC, Shanely RA, Luo B, Dew D, Meaney MP, Sha W. A commercialized dietary supplement alleviates joint pain in community adults: a double-blind, placebo-controlled community trial. *Nutr J*. 2013;12(1):154. doi:10.1186/1475-2891-12-154
39. Penna G, Mondaini N, Amuchastegui S, et al. Seminal plasma cytokines and chemokines in prostate inflammation: interleukin 8 as a predictive biomarker in chronic prostatitis/chronic pelvic pain syndrome and benign prostatic hyperplasia. *Europ Urology*. 2007;51(2):524–533.
40. Ponniah S, Arah I, Alexander RB. PSA is a candidate self-antigen in autoimmune chronic prostatitis/chronic pelvic pain syndrome. *Prostate*. 2000;44(1):49–54. doi:10.1002/1097-0045(20000615)44:1<49::aid-pros7>3.0.co;2-7

41. Prada-Arias M, Gómez-Veiras J, Salgado-Barreira Á, Vázquez JL, Montero-Sánchez M, Fernández-Lorenzo JR. Value of fibrinogen to discriminate appendicitis from nonspecific abdominal pain in preschool children. *Eur J Pediatr Surg*. 2020;30(4):357–363. doi:10.1055/s-0039-1692166
42. Prada-Arias M, Vázquez JL, Salgado-Barreira Á, Gómez-Veiras J, Montero-Sánchez M, Fernández-Lorenzo JR. Diagnostic accuracy of fibrinogen to differentiate appendicitis from nonspecific abdominal pain in children. *Am J Emerg Med*. 2017;35(1):66–70. doi:10.1016/j.ajem.2016.10.003
43. Radojčić MR, Thudium CS, Henriksen K, et al. Biomarker of extracellular matrix remodelling C1M and proinflammatory cytokine interleukin 6 are related to synovitis and pain in end-stage knee osteoarthritis patients. *Pain*. 2017;158(7):1254–1263. doi:10.1097/j.pain.0000000000000908
44. Ramanathan S, Douglas SR, Alexander GM, et al. Exosome microRNA signatures in patients with complex regional pain syndrome undergoing plasma exchange. *J Transl Med*. 2019;17(1):81. doi:10.1186/s12967-019-1833-3
45. Reed JL, Strait RT, Kachelmeyer AM, Byczkowski TL, Ho ML, Huppert JS. Biomarkers to distinguish surgical etiologies in females with lower quadrant abdominal pain. *Acad Emerg Med*. 2011;18(7):686–691. doi:10.1111/j.1553-2712.2011.01108.x
46. Rhee KY, Goetzl L, Unal R, Cierny J, Flood P. The relationship between plasma inflammatory cytokines and labor pain. *Anesth Analg*. 2015;121 (3):748–751. doi:10.1213/ane.0000000000000837
47. Ritz BW, Alexander GM, Nogusa S, et al. Elevated blood levels of inflammatory monocytes (CD14+ CD16+) in patients with complex regional pain syndrome. *Clin Exp Immunol*. 2011;164(1):108–117. doi:10.1111/j.1365-2249.2010.04308.x
48. Rocha AL, Vieira EL, Ferreira MC, Maia LM, Teixeira AL, Reis FM. Plasma brain-derived neurotrophic factor in women with pelvic pain: a potential biomarker for endometriosis? *Biomarker Med*. 2017;11(4):313–317. doi:10.2217/bmm-2016-0327
49. Sanchis J, Bosch X, Bodí V, et al. Combination of clinical risk profile, early exercise testing and circulating biomarkers for evaluation of patients with acute chest pain without ST-segment deviation or troponin elevation. *Heart*. 2008;94(3):311–315. doi:10.1136/hrt.2007.115626
50. Simon CB, Bishop MD, Wallace MR, et al. Circulating inflammatory biomarkers predict pain change following exercise-induced shoulder injury: findings from the biopsychosocial influence on shoulder pain preclinical trial. *J Pain*. 2023;24(8):1465–1477. doi:10.1016/j.jpain.2023.04.001
51. Thakkar B, Acevedo EO. BDNF as a biomarker for neuropathic pain: consideration of mechanisms of action and associated measurement challenges. *Brain Behav*. 2023;13(3):e2903. doi:10.1002/brb3.2903
52. Tofik R, Swärd P, Ekelund U, et al. Plasma pro-inflammatory cytokines, IgM-uria and cardiovascular events in patients with chest pain: a comparative study. *Scand J Clin Lab Invest*. 2015;75(8):638–645. doi:10.3109/00365513.2015.1057218
53. Wereszczynska-Siemiatkowska U, Dabrowski A, Siemiatkowski A, Mroczko B, Laszewicz W, Gabryelewicz A. Serum profiles of E-selectin, interleukin-10, and interleukin-6 and oxidative stress parameters in patients with acute pancreatitis and nonpancreatic acute abdominal pain. *Pancreas*. 2003;26(2):144–152. doi:10.1097/00006676-200303000-00010
54. Wu EB, Lumb P, Chambers JB, Crook MA. Plasma sialic acid and coronary artery atheromatous load in patients with stable chest pain. *Atherosclerosis*. 1999;145(2):261–266. doi:10.1016/s0021-9150(99)00074-x
55. Gerdle B, Bäckryd E, Falkenberg T, Lundström E, Ghafouri B. Changes in inflammatory plasma proteins from patients with chronic pain associated with treatment in an interdisciplinary multimodal rehabilitation program - an explorative multivariate pilot study. *Scand J Pain*. 2019;20 (1):125–138. doi:10.1515/sjpain-2019-0088
56. Goode AP, Hu D, George SZ, et al. Biomarker clusters differentiate phenotypes of lumbar spine degeneration and low back pain: the Johnston County Osteoarthritis Project. *Osteoarthr Cartil Open*. 2022;4(3). doi:10.1016/j.ocarto.2022.100270
57. Jönsson M, Gerdle B, Ghafouri B, et al. The inflammatory profile of cerebrospinal fluid, plasma, and saliva from patients with severe neuropathic pain and healthy controls-a pilot study. The inflammatory profile of cerebrospinal fluid, plasma, and saliva from patients with severe neuropathic pain and healthy controls-a pilot study. *BMC neuro*. 2021;22:6. doi:10.1186/s12868-021-00608-5
58. Karppinen J, Koivisto K, Ketola J, et al. Serum biomarkers for Modic changes in patients with chronic low back pain. *Eur Spine J*. 2021;30 (4):1018–1027. doi:10.1007/s00586-020-06713-z
59. Mann TN, Davis JH, Walzl G, et al. Candidate biomarkers to distinguish spinal tuberculosis from mechanical back pain in a tuberculosis endemic setting. *Front Immunol*. 2021;12:768040. doi:10.3389/fimmu.2021.768040
60. Paish HL, Baldock TE, Gillespie CS, et al. Chronic, active inflammation in patients with failed total knee replacements undergoing revision surgery. *J Orthop Res*. 2019;37(11):2316–2324. doi:10.1002/jor.24398
61. Sarchielli P, Alberti A, Vaianella L, et al. Chemokine levels in the jugular venous blood of migraine without aura patients during attacks. Comparative Study. *Headache*. 2004;44(10):961–968.
62. Scholl B, Bersinger NA, Kuhn A, Mueller MD. Correlation between symptoms of pain and peritoneal fluid inflammatory cytokine concentrations in endometriosis. *Gynecol Endocrinol*. 2009;25(11):701–706. doi:10.3109/09513590903159680
63. Sedighi M, Haghnegahdar A. Role of vitamin D3 in treatment of lumbar disc herniation--pain and sensory aspects: study protocol for a randomized controlled trial. *Trials*. 2014;15:373. doi:10.1186/1745-6215-15-373
64. Slouma M, Kharrat L, Tezegdenti A, et al. Pro-inflammatory cytokines in patients with low back pain: a comparative study. *Reumatol Clin*. 2023;19 (5):244–248. doi:10.1016/j.reumae.2022.07.002
65. Sugimoto C, Konno T, Wakao R, Fujita H, Fujita H, Wakao H. Mucosal-associated invariant T cell is a potential marker to distinguish fibromyalgia syndrome from arthritis. *PLoS One*. 2015;10(4):e0121124. doi:10.1371/journal.pone.0121124
66. Tay ML, Bolam SM, Monk AP, McGlashan SR, Young SW, Matthews BG. Better post-operative outcomes at 1-year follow-up are associated with lower levels of pre-operative synovitis and higher levels of IL-6 and VEGFA in unicompartmental knee arthroplasty patients. *Knee Surg Sports Traumatol Arthrosc*. 2023;31(10):4109–4116. doi:10.1007/s00167-023-07503-y
67. Jiang YH, Jhang JF, Hsu YH, Ho HC, Wu YH, Kuo HC. Urine cytokines as biomarkers for diagnosing interstitial cystitis/bladder pain syndrome and mapping its clinical characteristics. *Am J Physiol Renal Physiol*. 2020;318(6):F1391–F1399. doi:10.1152/ajprenal.00051.2020
68. Yücel Ç, Fırat Oğuz E, Er S, Balamir İ, Turhan T, Tez M. Diagnostic value of GCP-2/CXCL-6 and hs-CRP in the diagnosis of acute appendicitis. *Ulus Travma Acil Cerrahi Derg*. 2020;26(2):191–196. doi:10.14744/tjtes.2019.26270
69. Andersen T, Ueland T, Aukrust P, et al. Procollagen type 1 N-terminal propeptide is associated with adverse outcome in acute chest pain of suspected coronary origin. *Front Cardiovasc Med*. 2023;10:1191055. doi:10.3389/fcvm.2023.1191055
70. Aslam I, Perjar I, Shi XA, et al. Associations between biomarkers of joint metabolism, hand osteoarthritis, and hand pain and function: the Johnston County Osteoarthritis Project. *J Rheumatol*. 2014;41(5):938–944. doi:10.3899/jrheum.130904
71. Bihlet AR, Byrjalsen I, Bay-Jensen AC, et al. Associations between biomarkers of bone and cartilage turnover, gender, pain categories and radiographic severity in knee osteoarthritis. *Arthritis Res Ther*. 2019;21(1):203. doi:10.1186/s13075-019-1987-7

72. Bolgla LA, Gordon R, Sloan G, Pretlow LG, Lyon M, Fulzele S. Comparison of patella alignment and cartilage biomarkers in young adult females with and without patellofemoral pain: a Pilot Study. *Int J Sports Phys Ther*. 2019;14(1):46–54. doi:10.26603/ijspt20190046
73. Chahal J, Gomez-Aristizabal A, Shestopaloff K, et al. Bone marrow mesenchymal stromal cell treatment in patients with osteoarthritis results in overall improvement in pain and symptoms and reduces synovial inflammation. *Stem Cells Transl Med*. 2019;8(8):746–757. doi:10.1002/sctm.18-0183
74. Forni GL, Perrotta S, Giusti A, et al. Neridronate improves bone mineral density and reduces back pain in beta-thalassaemia patients with osteoporosis: results from a Phase 2, randomized, parallel-arm, open-label study. *Brit J Haematol*. 2012;158(2):274–282. doi:10.1111/j.1365-2141.2012.09152.x
75. Garcia-Alvarado FJ, Gonzalez-Martinez MDR, Jaramillo-Rodriguez Y, Delgado-Aguirre HA. Increased urinary concentration of C-terminal telopeptide of type ii collagen and pain by radiographic grade in women with knee osteoarthritis in Northeastern Mexico: a cross-sectional study. *Biores Open Access*. 2020;9(1):7–12. doi:10.1089/biores.2019.0003
76. Ghaffari S, Yaghoubi A, Baghernejad R, Sepehrvand N, Sokhanvar S, Haghjou AG. The value of serum osteoprotegerin levels in patients with angina like chest pain undergoing diagnostic coronary angiography. *Cardiol J*. 2013;20(3):261–267. doi:10.5603/CJ.2013.0071
77. Hider SL, Konstantinou K, Hay EM, Glossop J, Mattey DL. Inflammatory biomarkers do not distinguish between patients with sciatica and referred leg pain within a primary care population: results from a nested study within the ATLAS cohort. *BMC Musculoskelet Disord*. 2019;20(1):202. doi:10.1186/s12891-019-2604-2
78. Husain SF, Lam RWM, Hu T, et al. Locating the site of neuropathic pain in vivo using MMP-12-targeted magnetic nanoparticles. *Pain Res Manag*. 2019:9394715. doi:10.1155/2019/9394715
79. Ishijima M, Watari T, Naito K, et al. Relationships between biomarkers of cartilage, bone, synovial metabolism and knee pain provide insights into the origins of pain in early knee osteoarthritis. *Arthritis Res Ther*. 2011;13(1):R22. doi:10.1186/ar3246
80. Jacobs CA, Vranceanu AM, Thompson KL, Lattermann C. Rapid progression of knee pain and osteoarthritis biomarkers greatest for patients with combined obesity and depression: data from the osteoarthritis initiative. *Cartilage*. 2020;11(1):38–46. doi:10.1177/1947603518777577
81. Kalahasthi R, Bagepally BS, Barman T. Association between musculoskeletal pain and bone turnover markers in long-term Pb-exposed workers. *J Res Health Sci*. 2021;21(3):e00522. doi:10.34172/jrhs.2021.55
82. Leung YY, Huebner JL, Haaland B, Wong SBS, Kraus VB. Synovial fluid pro-inflammatory profile differs according to the characteristics of knee pain. *Osteoarthritis Cartilage*. 2017;25(9):1420–1427. doi:10.1016/j.joca.2017.04.001
83. Li H, Wang B, He L, Tao R, Shang S. Application of bone metabolic parameters in the diagnosis of growing pains. *J Clin Lab Anal*. 2022;36(2):e24184. doi:10.1002/jcla.24184
84. Meehan RT, Gill MT, Hoffman ED, et al. Ultrasound-guided injections of HYADD4 for knee osteoarthritis improves pain and functional outcomes at 3, 6, and 12 months without changes in measured synovial fluid, serum collagen biomarkers, or most synovial fluid biomarker proteins at 3 months. *J Clin Med*. 2023;12(17):25. doi:10.3390/jcm12175541
85. Port H, Nielsen SH, Madsen SF, et al. Extracellular matrix protein turnover markers are associated with axial spondyloarthritis-a comparison with postpartum women and other non-axial spondyloarthritis controls with or without back pain. *Arthritis Res Ther*. 2022;24(1):152. doi:10.1186/s13075-022-02839-1
86. Roy R, Stephens AJ, Daisy C, et al. Association of longitudinal changes in symptoms and urinary biomarkers in patients with urological chronic pelvic pain syndrome: a MAPP Research Network Study. *J Urol*. 2021;205(2):514–523. doi:10.1097/JU.0000000000001391
87. Ruff KJ, Morrison D, Duncan SA, Back M, Aydogan C, Theodosakis J. Beneficial effects of natural eggshell membrane versus placebo in exercise-induced joint pain, stiffness, and cartilage turnover in healthy, postmenopausal women. *Clin Interv Aging*. 2018;13:285–295. doi:10.2147/CIA.S153782
88. Selistre LFA, Goncalves GH, Vasilceac FA, et al. The relationship between urinary C-Telopeptide fragments of type II collagen, knee joint load, pain, and physical function in individuals with medial knee osteoarthritis. *Braz J Phys Ther*. 2021;25(1):62–69. doi:10.1016/j.bjpt.2020.02.002
89. Stakos DA, Tziakas DN, Chalikias G, Mitrousi K, Tsigalou C, Boudoulas H. Chest pain in patients with arterial hypertension, angiographically normal coronary arteries and stiff aorta: the aortic pain syndrome. *Hellenic J Cardiol*. 2013;54(1):25–31.
90. van Berkel AC, van Spil WE, Schiphof D, et al. Associations between biomarkers of matrix metabolism and inflammation with pain and fatigue in participants suspected of early Hip and or knee osteoarthritis: data from the CHECK study. *Osteoarthritis Cartilage*. 2022;30(12):1640–1646. doi:10.1016/j.joca.2022.08.013
91. Zhou K, Li YJ, Soderblom EJ, et al. A "best-in-class" systemic biomarker predictor of clinically relevant knee osteoarthritis structural and pain progression. *Sci Adv*. 2023;9(4):eabq5095. doi:10.1126/sciadv.abq5095
92. Le Roy B, Martin-Krumm C, Pinol N, Dutheil F, Trousselard M. Human challenges to adaptation to extreme professional environments: a systematic review. *Neurosci Biobehav Rev*. 2023;146:105054. doi:10.1016/j.neubiorev.2023.105054