Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Physiology

Sex differences and mechanisms of muscle pain

Luis F Queme[1] and Michael P Jankowski[1,2]

Clinical conditions resulting in musculoskeletal pain show important sex differences in both prevalence and degree of functional disability. The underlying mechanisms for these distinctions in pain manifestation are not fully known. However, recent preclinical studies have shown at the primary afferent level that males and females present fundamental differences in their peripheral response properties and injury-related gene expression patterns that may underlie observed afferent sensitization. At the spinal cord level, studies in various models of pain suggest important roles for the immune system, glutamate signaling and hormones in modulating sex differences. While preclinical studies have been able to characterize some of the basic underlying molecular mechanisms of sex differences in muscle pain, human studies have relied mainly on functional brain imaging studies to explain differences. Further complicating our understanding of how sex influences muscle pain is the notion that the type of injury sustained, or clinical condition may differentially activate distinct mechanisms of muscle pain development in males versus females. More research is necessary to better understand how the sexes differ in their perception of muscle pain. This review highlights recent advances in both human and animal studies of sex differences in muscle pain.

Addresses
[1] Department of Anesthesia, Division of Pain Management, Cincinnati Children's Medical Center, 3333 Burnet Ave, Cincinnati, OH, 45229, USA
[2] Department of Pediatrics, University of Cincinnati, College of Medicine, 3333 Burnet Ave, Cincinnati, OH, 45229, USA

Corresponding author:
Jankowski, Michael P (michael.jankowski@cchmc.org)

Current Opinion in Physiology 2019, **11**:1–6

This review comes from a themed issue on **Physiology of pain**

Edited by **Lucy F Donaldson** and **Cheryl L Stucky**

For a complete overview see the Issue and the Editorial

Available online 2nd April 2019

https://doi.org/10.1016/j.cophys.2019.03.006

2468-8673/© 2019 Elsevier Ltd. All rights reserved.

Introduction

Many clinical conditions that include musculoskeletal pain as a component, such as fibromyalgia, complex regional pain syndrome (CRPS), low back pain or myofascial pain have a higher prevalence in females [1–5]. Women also often report greater functional impairment compared to men [6]. While the sex differences in the epidemiology of musculoskeletal pain have been known for quite some time, only recently have clinical and preclinical studies begun to focus on these differences in multiple muscle pain conditions or in animal models. Earlier studies have often only focused on males and few have centered on the basic mechanisms of musculoskeletal pain or its transition to chronic pain.

In this review, we will focus on recently reported findings from patients living with painful musculoskeletal conditions or in healthy human subjects undergoing experimental muscle pain. This will be paired with a review of recent literature on animal models of myalgia. We will highlight recent advances in our basic understanding of sex differences in muscle pain mechanisms and emphasize areas in need of further investigation.

Sex differences in human musculoskeletal pain

In humans, there are sex differences in pain perception at multiple levels, but whether men or women display distinctions in pain appears to be context-dependent. In general, women are often reported to have reduced tolerance and increased responses to painful stimuli [7–10]. For example, in a study with patients experiencing shoulder pain, women reported greater clinical pain and had enhanced sensitivity to pressure pain [11]. Interestingly while both men and women can report the same musculoskeletal pain severity, women often report a significantly higher level of activity and pain acceptance. Men experience higher fear of movement, and mood disturbances tied to lower activity levels [12]. Recent reports also show differences in how the experience of pain is remembered. Males, but not females, exhibit increased pain hypersensitivity if placed in a context that is similar to previous painful experiences, and this may be mediated by testosterone [13••].

Experimental musculoskeletal pain in human subjects has also provided context-dependent results. Studies analyzing sex differences in humans undergoing delayed onset muscle soreness have not found any significant differences between men and women [14•,15]. Other experimental investigations using hypertonic saline-evoked muscle pain, however, have reported increased pressure point thresholds in males but not in females [16]. In a model of endotoxemia, one way to induce widespread inflammatory pain, a study revealed decreased pressure pain thresholds (PPT) in women at baseline, but no sex differences after inflammation [17].

This variability could be due to many factors. Age may be one important element as recent work found no differences in pain sensitivity or pain affect between older males and females [18•], in contrast with the findings reported in younger adults [6,10,11,19]. Another element commonly observed with myalgia is an altered cardiovascular response to muscle contraction; also referred to as the exercise pressor reflex (EPR). Women have been reported to have a blunted metaboreflex compared to men under normal conditions [20]. However, some studies have shown increased vasomotor responses and pain in females after intramuscular injection of hypertonic saline [21].

From a mechanistic point of view, variations could also be tied to sex differences in brain activation patterns induced by muscle pain as previously reported using fMRI. Significant changes in signal intensity in the mid-cingulate cortex and dorsolateral prefrontal cortex, occur in a sex-dependent dimorphic pattern, suggesting important differences in the emotional processing of pain [22]. While sex differences have been detected in healthy human subjects and in chronic muscle pain patients, more studies are necessary to elucidate the underlying mechanisms behind these phenomena in both the periphery and central nervous system. Preclinical animal studies are also crucial to enhance our mechanistic understanding of sex differences in muscle pain, in addition to clinical investigations.

Sex differences in preclinical models of muscle pain

Only recently have preclinical studies begun to dissect the mechanisms of muscle pain and the role of sex on its expression. Like human studies, preclinical assessments also appear to provide context-specific results regarding sex and myalgia. Earlier reports analyzing inflammatory models of muscle pain have not detected sex differences in evoked hypersensitivity [23]. This was not the case in other models, where female mice that were exposed to muscle fatigue show increased response to mechanical stimulation of the paw; an effect that was not present in ovariectomized mice [24]. Interestingly, when muscle inflammation was paired with fatigue, sex differences were not observed [24]. In contrast, in a more recent mouse model of localized fatigue paired with a low-pH (5.0) injections, mechanical hyperalgesia was observed in both male and female mice. However, males resolved by day 14 while females did not return to baseline levels until 42 days post insult. Furthermore, males required the insult to occur immediately after fatigue induction to develop mechanical hyperalgesia while females developed hypersensitivity even when the insult was delivered 24 hours after muscle fatigue [25]. Estrogen did not appear to mediate these outcomes as ovariectomized females behaved similar to their intact counterparts [26•]. Macrophages, however, may be part of the sensitization process as local depletion using clondronate liposomes, prevented the development of mechanical sensitization [26•]. Sex differences have also been observed in a mouse model of CRPS [27]. In this study, female mice that experience a fracture injury and subsequent casting displayed lower nociceptive thresholds compared to males. Female mice also showed exaggerated signs of motor dysfunction, and latent sensitization.

Differences in sensitization likely involve many components of the pain pathway. Group III/IV primary afferents are the main transducers of nociceptive signals from muscle tissue. Their sensory detection capabilities are varied as they can respond to mechanical stimuli in the noxious and non-noxious ranges, can have responsiveness to heat or cold, and can be activated by chemical stimuli that often include varying combinations of lactate, ATP and protons. These afferents can be single modality units as well as polymodal [28]. The chemosensory functions of these afferents in particular, have been frequently associated with sensations of fatigue or pain [28,29]. Furthermore, primary muscle afferents also comprise the sensory arm of the exercise pressor reflex [30,31].

Electrophysiological analysis of uninjured primary muscle afferents shows that certain response properties are distinct between males and females. For example, females appear to have greater numbers of mechanically sensitive group III/IV muscle afferents at baseline, which often display increased firing to mechanical deformation of the muscles after stimulation with metabolite mixtures containing lactate, ATP and protons. In addition, female afferents also seem to respond to heat stimuli with higher firing rates [32••]. Under certain injury conditions such as ischemia with reperfusion injury (I/R), female primary muscle afferents display higher firing to cold stimuli while males do not [32••,33].

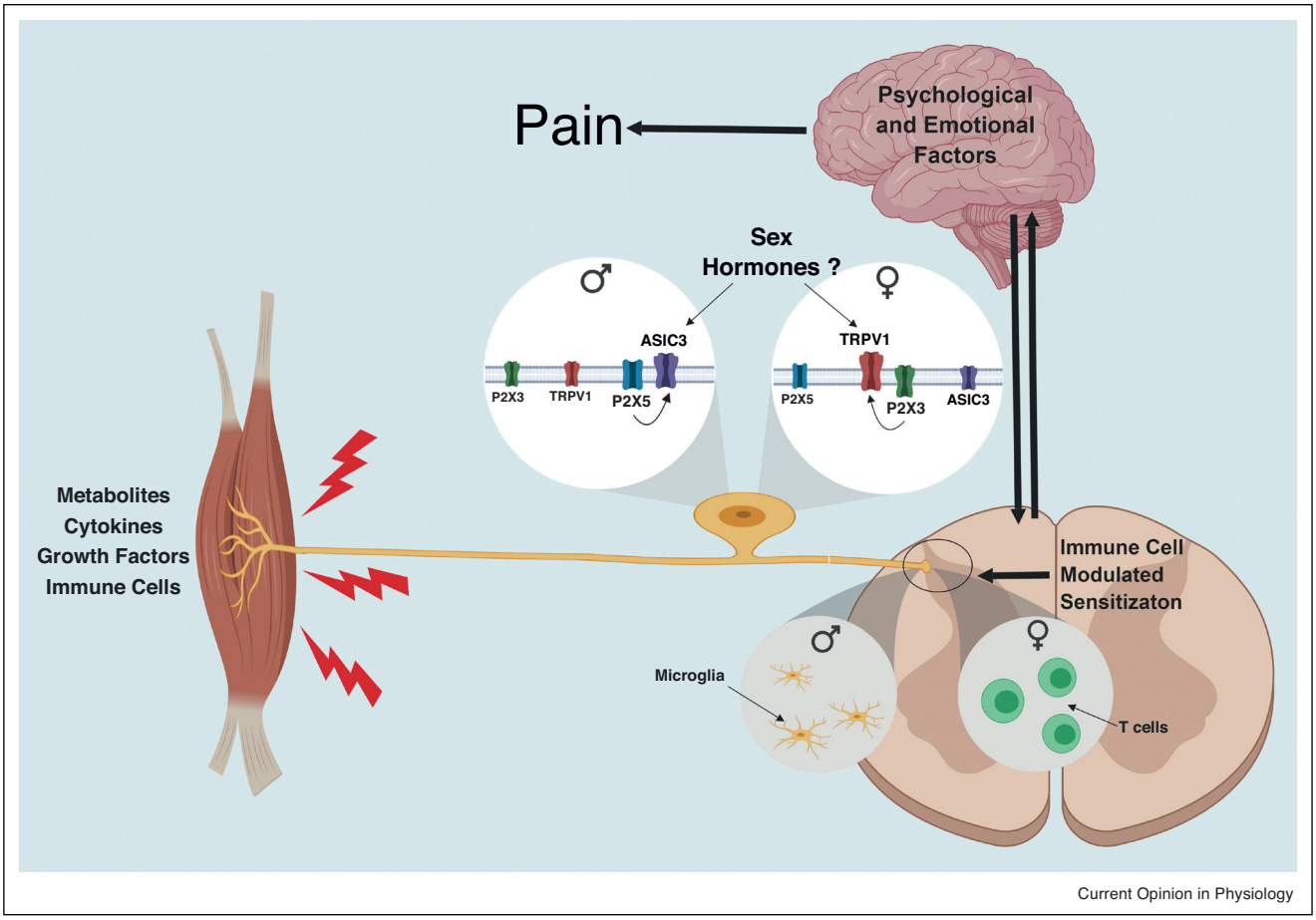
Gene expression analysis of DRGs after ischemic muscle injury, for example, suggests that females display specific upregulation of the heat and proton sensing channel, transient receptor potential vanilloid type 1 (TRPV1) and the cold responsive receptor, TRP melastatin 8 (TRPM8) [32••]. Males appear to distinctly upregulate acid sensing ion channel 3 (ASIC3). Both males and females upregulate the ATP receptors, P2X3 and P2X5 after muscle ischemia [31,33,34]. Each of these changes in gene expression has been correlated with exacerbated pain related-behaviors [33]. ASIC3 has been linked to pain sensitization in multiple models of muscle pain [23,26•,33,35] and it has been suggested that P2X5 can modulate the pH sensitivity of ASIC3 [36]. TRPV1 has also been associated with hypersensitivity in multiple animal models, including, inflammation [37••,38,39], incisional pain [40,41], delayed onset muscle soreness [42,43], and ischemic myalgia [44,45]. In trigeminal neurons, it has been reported to interact with P2X3 in order to

modulate afferent sensitization [46]. While results from ischemic injuries suggest a role for TRPV1 in female muscle afferent sensitization, the context of the injury again may be an important factor by which hypersensitivity is induced. One report evaluating mechanical hypersensitivity after carrageenan-induced muscle inflammation in male mice suggested that TRPV1 modulates mechanical sensitivity [38]. Nevertheless, results strongly imply that even before injury, male and female primary muscle afferents are fundamentally different. Moreover, while both sexes experience sensitization and behavioral changes associated with muscle pain after injury, the underlying molecular mechanisms for altered nociception are also likely distinct.

Sex differences in mechanisms of muscle pain development are not only observed at the DRG level but are also found in the spinal cord. This is important as primary muscle afferents have been known for decades to be highly efficient at evoking central sensitization. Repetitive stimulation of the gastrocnemius nerve causes greater long-term potentiation (LTP) of C fiber-evoked field potentials in the spinal dorsal horn compared with stimulation of a cutaneous nerve [48]. Furthermore, compared to cutaneous afferents, muscle sensory neurons are more effective at increasing the excitability of spinal flexion reflexes [49,50]. In models of CRPS, the levels of the glutamate receptor NR2b were decreased in males but not in females and this was thought to modulate central sensitization [27].

In a model of peripheral nerve injury, other important sex differences involving the immune system have been shown to regulate the development of pain. In both male and female mice, microglia proliferate in the spinal cord after peripheral nerve injury, but only males use microglia as mediators for the development of prolonged

Figure 1



Sex-related mechanisms of muscle pain.
In certain diseases or upon injury, muscle tissue releases a variety of metabolites, cytokines and grow factors that can be accompanied by immune cell infiltration. These signals are paired with differential gene expression patterns and receptor interactions between males and females in the DRGs. Meanwhile in the spinal cord, the increased signals from muscle afferents are possibly modulated by increased immune reactivity of microglia in males and T-cells in females. The perception of pain in the brain can be further influenced by sex-specific psychological and emotional factors and may lead to the distinct sensations of pain in men versus women.

hypersensitivity [51••], possibly through a mechanism dependent on the purinergic receptor, P2X4 [52]. Females do not upregulate P2X4 receptors but use a microglia-independent pathway to mediate pain hypersensitivity, potentially involving T cells. The unique functions of microglia may be applicable to muscle pain as studies in a rat model of chronic myositis report increased microglia immunoreactivity in the spinal cord associated with mechanical hyperalgesia that was effectively reversed by gabapentin administration [53]. Glial cells have also been linked to mechanical hypersensitivity in a rat model of low back pain as glial inhibitors, minocycline, and fluorocitrate were effective at preventing latent spinal sensitization [54].

Microglia may also influence the observed sex differences in morphine-induced analgesia. A study using peripheral administration of LPS to activate periaqueductal gray (PAG) microglia showed that greater activation in females compared to males was accompanied by increased transcriptional levels of interleukin 1β (IL-1β) and a significant rightward shift in the morphine dose–response curve [55]. In the same study, naloxone effectively potentiated morphine anti-nociception in females such that no sex differences in were observed. Other research on masseter muscle myositis showed that only males upregulated the μ-opioid receptor in the trigeminal ganglion and that orchidectomy prevented this upregulation [47]. Overall, these findings support a crucial role for immune cells in the sex-dependent mechanisms of muscle pain development.

There is further evidence suggesting that sex hormones can directly play a role in pain sensitization. In many cases, the effects mediated by hormones can be striking, as exemplified by a recent report showing that male rodents can experience testosterone-mediated conditioned pain hypersensitivity. This effect could also be translated to humans [13••]. One report has shown in a mouse model of reserpine-induced widespread muscle pain that mechanical hyperalgesia was enhanced by ovariectomy and partially rescued in a dose-dependent fashion by the administration of 17β-estradiol [56]. In contrast, testosterone has been shown to regulate gene expression in a mouse model of CFA-induced masseter muscle inflammation. Female and orchiectomized male rats displayed TRPV1 upregulation after inflammation, and replacement of testosterone in the orchiectomized males was effective in preventing the observed upregulation of TRPV1 [37••]. These data may help explain results observed in ischemic muscle pain whereby TRPV1 may play a stronger role in hypersensitivity in females than males [32••]. Collectively, this suggests multiple sex differences in mechanisms of peripheral and central sensitization after muscle injury.

Final considerations

Musculoskeletal pain is a complex phenomenon, and sex differences have been reported at multiple steps in the nociceptive signaling pathway. Primary sensory afferents, spinal cord modulation, and brain processing all present variations between sexes (Figure 1). These need to be taken into account in future research aimed at developing therapies targeting the complex pathologies that drive muscle pain between men and women.

Conflict of interest statement

Nothing declared.

Funding sources

This work was supported by grant to MPJ from the NIH/NIAMS (R01AR064551).

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Wolfe F, Walitt B, Perrot S, Rasker JJ, Hauser W: **Fibromyalgia diagnosis and biased assessment: sex, prevalence and bias**. *PLoS One* 2018, **13**:e0203755.
2. Elsamadicy AA, Yang S, Sergesketter AR, Ashraf B, Charalambous L, Kemeny H, Ejikeme T, Ren X, Pagadala P, Parente B, Xie J, Lad SP: **Prevalence and cost analysis of complex regional pain syndrome (CRPS): a role for neuromodulation**. *Neuromodulation: J Int Neuromodulation Soc* 2018, **21**:423-430.
3. Meucci RD, Fassa AG, Faria NM: **Prevalence of chronic low back pain: systematic review**. *Rev Saude Publica* 2015, **49**.
4. Cimas M, Ayala A, Sanz B, Agullo-Tomas MS, Escobar A, Forjaz MJ: **Chronic musculoskeletal pain in European older adults: cross-national and gender differences**. *Eur J Pain* 2018, **22**:333-345.
5. Wijnhoven HA, de Vet HC, Picavet HS: **Prevalence of musculoskeletal disorders is systematically higher in women than in men**. *Clin J Pain* 2006, **22**:717-724.
6. Fillingim RB, King CD, Ribeiro-Dasilva MC, Rahim-Williams B, Riley JL 3rd: **Sex, gender, and pain: a review of recent clinical and experimental findings**. *J Pain* 2009, **10**:447-485.
7. Bueno CH, Pereira DD, Pattussi MP, Grossi PK, Grossi ML: **Gender differences in temporomandibular disorders in adult populational studies: a systematic review and meta-analysis**. *J Oral Rehabil* 2018, **45**:720-729.
8. Sorge RE, Totsch SK: **Sex differences in pain**. *J Neurosci Res* 2017, **95**:1271-1281.
9. Mogil JS: **Sex differences in pain and pain inhibition: multiple explanations of a controversial phenomenon**. *Nat Rev Neurosci* 2012, **13**:859-866.
10. Bartley EJ, Fillingim RB: **Sex differences in pain: a brief review of clinical and experimental findings**. *Br J Anaesth* 2013, **111**:52-58.
11. Kindler LL, Valencia C, Fillingim RB, George SZ: **Sex differences in experimental and clinical pain sensitivity for patients with shoulder pain**. *Eur J Pain* 2011, **15**:118-123.
12. Rovner GS, Sunnerhagen KS, Bjorkdahl A, Gerdle B, Borsbo B, Johansson F, Gillanders D: **Chronic pain and sex-differences; women accept and move, while men feel blue**. *PLoS One* 2017, **12**:e0175737.
13. •• Martin LJ, Acland EL, Cho C, Gandhi W, Chen D, Corley E, Kadoura B, Levy T, Mirali S, Tohyama S *et al.*: **Male-specific conditioned pain hypersensitivity in mice and humans**. *Curr Biol* 2019, **29**:192-201.e4.

This study shows for the first time that male mice display pain hypersensitivity when exposed to a context associated with a noxious stimulus. This mechanism seems to be mediated by testosterone and the phenomenon is translatable to human subjects.

14. • Dannecker EA, Liu Y, Rector RS, Thomas TR, Fillingim RB, Robinson ME: **Sex differences in exercise-induced muscle pain and muscle damage**. *J Pain* 2012, **13**:1242-1249.
The authors use an eccentric exercise to induce muscle damage and monitor various biochemical and behavioral parameters to confirm muscle damage. Women reported moderately lower and less frequent muscle pain than men and also showed smaller increases in myoglobin and arm girth after exercise when compared to men.

15. Dannecker EA, Hausenblas HA, Kaminski TW, Robinson ME: **Sex differences in delayed onset muscle pain**. *Clin J Pain* 2005, **21**:120-126.

16. Arendt-Nielsen L, Sluka KA, Nie HL: **Experimental muscle pain impairs descending inhibition**. *Pain* 2008, **140**:465-471.

17. Wegner A, Elsenbruch S, Rebernik L, Roderigo T, Engelbrecht E, Jäger M, Engler H, Schedlowski M, Benson S: **Inflammation-induced pain sensitization in men and women: does sex matter in experimental endotoxemia?** *Pain* 2015, **156**:1954-1964.

18. • Monroe TB, Fillingim RB, Bruehl SP, Rogers BP, Dietrich MS, Gore JC, Atalla SW, Cowan RL: **Sex differences in brain regions modulating pain among older adults: a cross-sectional resting state functional connectivity study**. *Pain Med* 2018, **19**:1737-1747.
In this report, females exhibited greater association between thermal pain sensitivity and resting state functional conectivity indicating a basal difference in nociceptive processing between males and females.

19. Gupta A, Mayer EA, Fling C, Labus JS, Naliboff BD, Hong JY, Kilpatrick LA: **Sex-based differences in brain alterations across chronic pain conditions**. *J Neurosci Res* 2017, **95**:604-616.

20. Jarvis SS, VanGundy TB, Galbreath MM, Shibata S, Okazaki K, Reelick MF, Levine BD, Fu Q: **Sex differences in the modulation of vasomotor sympathetic outflow during static handgrip exercise in healthy young humans**. *Am J Physiol Regul Integr Comp Physiol* 2011, **301**:R193-R200.

21. Lei J, You HJ: **Variation of pain and vasomotor responses evoked by intramuscular infusion of hypertonic saline in human subjects: influence of gender and its potential neural mechanisms**. *Brain Res Bull* 2012, **87**:564-570.

22. Henderson LA, Gandevia SC, Macefield VG: **Gender differences in brain activity evoked by muscle and cutaneous pain: a retrospective study of single-trial fMRI data**. *Neuroimage* 2008, **39**:1867-1876.

23. Sluka KA, Radhakrishnan R, Benson CJ, Eshcol JO, Price MP, Babinski K, Audette KM, Yeomans DC, Wilson SP: **ASIC3 in muscle mediates mechanical, but not heat, hyperalgesia associated with muscle inflammation**. *Pain* 2007, **129**:102-112.

24. Sluka KA, Rasmussen LA: **Fatiguing exercise enhances hyperalgesia to muscle inflammation**. *Pain* 2010, **148**:188-197.

25. Gregory NS, Gibson-Corley K, Frey-Law L, Sluka KA: **Fatigue-enhanced hyperalgesia in response to muscle insult: induction and development occur in a sex-dependent manner**. *Pain* 2013, **154**:2668-2676.

26. • Gregory NS, Brito RG, Fusaro MC, Sluka KA: **ASIC3 is required for development of fatigue-induced hyperalgesia**. *Mol Neurobiol* 2016, **53**:1020-1030.
In this work, the authors use a combination or electrically induced contractions and low pH injections to induce fatigue related hyperalgesia. ASIC3$^{-/-}$ mice and mice treated with ASIC3 blockers did not develop fatigue enhanced hyperalgesia. No sex differences in fatigue enhanced hyperalgesia were observed.

27. Tajerian M, Sahbaie P, Sun Y, Leu D, Yang HY, Li W, Huang TT, Kingery W, David Clark J: **Sex differences in a murine model of complex regional pain syndrome**. *Neurobiol Learn Mem* 2015, **123**:100-109.

28. Jankowski MP, Rau KK, Ekmann KM, Anderson CE, Koerber HR: **Comprehensive phenotyping of group III and IV muscle afferents in mouse**. *J Neurophysiol* 2013, **109**:2374-2381.

29. Pollak KA, Swenson JD, Vanhaitsma TA, Hughen RW, Jo D, White AT, Light KC, Schweinhardt P, Amann M, Light AR: **Exogenously applied muscle metabolites synergistically evoke sensations of muscle fatigue and pain in human subjects**. *Exp Physiol* 2014, **99**:368-380.

30. Kaufman MP: **The exercise pressor reflex in animals**. *Exp Physiol* 2012, **97**:51-58.

31. Queme LF, Ross JL, Lu P, Hudgins RC, Jankowski MP: **Dual modulation of nociception and cardiovascular reflexes during peripheral ischemia through P2Y1 receptor-dependent sensitization of muscle afferents**. *J Neurosci* 2016, **36**:19-30.

32. •• Ross JL, Queme LF, Lamb JE, Green KJ, Jankowski MP: **Sex differences in primary muscle afferent sensitization following ischemia and reperfusion injury**. *Biol Sex Differ* 2018, **9**:2.
Using a muscle ischemia with reperfusion injury model, male and female mice were shown to upregulate different receptors in the DRGs that may underlie peripheral sensitization. The study indicates there may be unique genetic mechanisms in nociceptors underlying muscle pain development between males and females.

33. Ross JL, Queme LF, Cohen ER, Green KJ, Lu P, Shank AT, An S, Hudgins RC, Jankowski MP: **Muscle IL1beta drives ischemic myalgia via ASIC3-mediated sensory neuron sensitization**. *J Neurosci* 2016, **36**:6857-6871.

34. Ross JL, Queme LF, Lamb JE, Green KJ, Ford ZK, Jankowski MP: **Interleukin 1beta inhibition contributes to the antinociceptive effects of voluntary exercise on ischemia/reperfusion-induced hypersensitivity**. *Pain* 2018, **159**:380-392.

35. Molliver DC, Immke DC, Fierro L, Pare M, Rice FL, McCleskey EW: **ASIC3, an acid-sensing ion channel, is expressed in metaboreceptive sensory neurons**. *Mol Pain* 2005, **1**:35.

36. Birdsong WT, Fierro L, Williams FG, Spelta V, Naves LA, Knowles M, Marsh-Haffner J, Adelman JP, Almers W, Elde RP, McCleskey EW: **Sensing muscle ischemia: coincident detection of acid and ATP via interplay of two ion channels**. *Neuron* 2010, **68**:739-749.

37. •• Bai X, Zhang X, Zhou Q: **Effect of testosterone on TRPV1 expression in a model of orofacial myositis pain in the rat**. *J Mol Neurosci: MN* 2018, **64**:93-101.
In a rat model of CFA-induced inflammatory myositis, the authors report sex related effects on TRPV1 upregulation in the trigeminal ganglion. The study shows how hormones may differentially regulate the role of this receptor in males and females during myositis.

38. Walder RY, Radhakrishnan R, Loo L, Rasmussen LA, Mohapatra DP, Wilson SP, Sluka KA: **TRPV1 is important for mechanical and heat sensitivity in uninjured animals and development of heat hypersensitivity after muscle inflammation**. *Pain* 2012, **153**:1664-1672.

39. Yu L, Yang F, Luo H, Liu FY, Han JS, Xing GG, Wan Y: **The role of TRPV1 in different subtypes of dorsal root ganglion neurons in rat chronic inflammatory nociception induced by complete Freund's adjuvant**. *Mol Pain* 2008, **4**:61.

40. Banik RK, Brennan TJ: **Trpv1 mediates spontaneous firing and heat sensitization of cutaneous primary afferents after plantar incision**. *Pain* 2009, **141**:41-51.

41. Pogatzki-Zahn EM, Shimizu I, Caterina M, Raja SN: **Heat hyperalgesia after incision requires TRPV1 and is distinct from pure inflammatory pain**. *Pain* 2005, **115**:296-307.

42. Ota H, Katanosaka K, Murase S, Kashio M, Tominaga M, Mizumura K: **TRPV1 and TRPV4 play pivotal roles in delayed onset muscle soreness**. *PLoS One* 2013, **8**:e65751.

43. Fujii Y, Ozaki N, Taguchi T, Mizumura K, Furukawa K, Sugiura Y: **TRP channels and ASICs mediate mechanical hyperalgesia in models of inflammatory muscle pain and delayed onset muscle soreness**. *Pain* 2008, **140**:292-304.

44. Ristoiu V, Shibasaki K, Uchida K, Zhou Y, Ton BH, Flonta ML, Tominaga M: **Hypoxia-induced sensitization of transient receptor potential vanilloid 1 involves activation of hypoxia-inducible factor-1 alpha and PKC**. *Pain* 2011, **152**:936-945.

45. Hillery CA, Kerstein PC, Vilceanu D, Barabas ME, Retherford D, Brandow AM, Wandersee NJ, Stucky CL: **Transient receptor**

potential vanilloid 1 mediates pain in mice with severe sickle cell disease. *Blood* 2011, **118**:3376-3383.

46. Saloman JL, Chung MK, Ro JY: **P2X(3) and TRPV1 functionally interact and mediate sensitization of trigeminal sensory neurons**. *Neuroscience* 2013, **232**:226-238.

47. Zhang X, Zhang Y, Asgar J, Niu KY, Lee J, Lee KS, Schneider M, Ro JY: **Sex differences in μ-opioid receptor expression in trigeminal ganglia under a myositis condition in rats**. *Eur J Pain* 2014, **18**:151-161.

48. Zhang J, Hoheisel U, Klein T, Magerl W, Mense S, Treede RD: **High-frequency modulation of rat spinal field potentials: effects of slowly conducting muscle vs. skin afferents**. *J Neurophysiol* 2016, **115**:692-700.

49. Wall PD, Woolf CJ: **Muscle but not cutaneous C-afferent input produces prolonged increases in the excitability of the flexion reflex in the rat**. *J Physiol* 1984, **356**:443-458.

50. Woolf CJ: **Central sensitization: implications for the diagnosis and treatment of pain**. *Pain* 2011, **152(Suppl. 3)**:S2-15.

51. •• Sorge RE, Mapplebeck JC, Rosen S, Beggs S, Taves S, Alexander JK, Martin LJ, Austin JS, Sotocinal SG, Chen D *et al.*: **Different immune cells mediate mechanical pain hypersensitivity in male and female mice**. *Nat Neurosci* 2015, **18**:1081-1083.

The authors report that after peripheral injury, male mice show increased microglial activity in the spinal cord while female mice seem to modulate pain via a T-cell-dependent mechanism. Data suggest unique sex-specific roles of the immune system in modulating pain.

52. Mapplebeck JC, Beggs S, Salter MW: **Sex differences in pain: a tale of two immune cells**. *Pain* 2016, **157(Suppl. 1)**:S2-S6.

53. Rosa AS, Freitas MF, Rocha IR, Chacur M: **Gabapentin decreases microglial cells and reverses bilateral hyperalgesia and allodynia in rats with chronic myositis**. *Eur J Pharmacol* 2017, **799**:111-117.

54. Zhang J, Mense S, Treede RD, Hoheisel U: **Prevention and reversal of latent sensitization of dorsal horn neurons by glial blockers in a model of low back pain in male rats**. *J Neurophysiol* 2017, **118**:2059-2069.

55. Doyle HH, Eidson LN, Sinkiewicz DM, Murphy AZ: **Sex differences in microglia activity within the periaqueductal gray of the rat: a potential mechanism driving the dimorphic effects of morphine**. *J Neurosci* 2017, **37**:3202-3214.

56. Hernandez-Leon A, De la Luz-Cuellar YE, Granados-Soto V, Gonzalez-Trujano ME, Fernandez-Guasti A: **Sex differences and estradiol involvement in hyperalgesia and allodynia in an experimental model of fibromyalgia**. *Horm Behav* 2018, **97**:39-46.