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TITLE: Targeting the Gut Microbiome to Treat Post-Traumatic Osteoarthritis

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14. ABSTRACT

While osteoarthritis (OA) is a debilitating condition with no disease modifying treatments, the gut microbiome may play a role in its development and progression. The establishment of a disease modifying treatment of OA has immense ramifications, including improved quality of life, lowered economic burden of treatment, and increased productivity of patients with OA. The purpose of this project is to study the pathogenic role of the microbiome in the development of OA as well as to develop microbiome-based treatments of the disease. Fecal microbiota transplants (FMTs) will be used to examine the causal relationship between microbiome dysbiosis that may develop in veterans diagnosed with post-traumatic osteoarthritis (PTOA) and belonging to the Military and Veteran Microbiome: Consortium for Research and Education (MVM-CoRE). Treatment of PTOA with microbiome pre and probiotics has begun and we find the dietary supplement hydrolyzed hylaline cartilage (hHC) to have protective effects on cartilage degeneration in a mouse model of PTOA. Despite institutional shutdowns caused by the COVID-19 pandemic, significant progress has been made on the first two objectives of this project, with progress expected to continue apace on all objectives.

15. SUBJECT TERMS

Osteoarthritis (OA); Posttraumatic osteoarthritis (PTOA); Gut microbiome; Fecal microbiota transplant (FMT); Military and Veteran Microbiome: Consortium for Research and Education (MVM-CoRE); Hydrolyzed hyaline cartilage (hHC); Destabilization of the medial meniscus (DMM); Peptococcaceae *rc4-4*; Anaeroplasmataceae; Firmicutes; Tenericutes; Cartilage; Chondrocyte; Synovium; Tumor Necrosis Factor-alpha (TNF)

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
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TABLE OF CONTENTS

1. Introduction

This project is focused on 1) studying the pathogenic role of the gut microbiome in the development of osteoarthritis (OA), and 2) developing approaches that target the gut microbiome to treat posttraumatic OA (PTOA), a disease that does not currently have an accepted disease modifying treatment. Our first objective is to study the microbiome dysbiosis that develops in a mouse model of PTOA and military veterans that have been diagnosed with PTOA and have been recruited into the Military and Veteran Microbiome: Consortium for Research and Education (MVM-CoRE). To prove the causal role of this dysbiosis, fecal microbiota transplants (FMTs) are planned (mouse to mouse or human to mouse), and specific potentially pathogenic microbial species will be gavaged to pinpoint taxa that are PTOA-accelerating. Our second objective is to build on or historical work studying dietary supplements as disease modifiers for OA by examining the impact of daily supplementation with hydrolyzed hyaline cartilage (hHC) on PTOA progression and on the gut microbiome. Again, FMTs between supplemented and non-supplemented mice will test the causal role of the gut community in any joint protection that is observed, and specific microbial species will be studies as active participants in the protective effects. Finally, in the third objective, we will focus on developing pre- and probiotic combinations for dietary supplements involving hHC and taxa identified in the second objective that promote joint protection and decelerated PTOA progression. Multiple dosing regimens will be tested, with the most protective combinations studies with deep analysis of the gut microbiome using cutting edge metagenomic, metatranscriptomic and metabolomic profiling to drill down on specific molecular fractions that are contributing to disease modification that we document. We have completed one year of work on this project, and despite a major interruption by the COVID-19 pandemic, we have made substantial progress in the first and second objectives. This progress is described below in the various sections of this report.

Note: This report provided information on the first year of a partnering-PI project, and the specific activity for each aspect of progress is attributed to the contributing PI (CU for Zuscik, UR for Gill). In general, all OA analysis, animal work, and human work occurs at CU. All analysis of gut microbiome and microbiology work to expand and maintain taxa of interest is performed at UR.

2. Key Words

Osteoarthritis (OA)
Posttraumatic osteoarthritis (PTOA)
Gut microbiome
Fecal microbiota transplant (FMT)
Military and Veteran Microbiome: Consortium for Research and Education (MVM-CoRE)
Hydrolyzed hyaline cartilage (hHC)
Destabilization of the medial meniscus (DMM)
Peptococcaceae *rc4-4*
Anaeroplasmataceae
Firmicutes
Tenericutes
Cartilage
Chondrocyte
Synovium
Tumor Necrosis Factor-alpha (TNF)

3. Accomplishments

- What were the major goals of the project?

The major goals of the project are 1) to define the role of the gut microbiome in PTOA, 2) to establish that hHC-induced chondroprotection in PTOA is due to effects on the gut microbiome, and 3) to test the efficacy of

combined pre- and pro-biotic strategies to treat PTOA. Our aim is to accomplish these goals using state-of-the-art approaches and methods, culminating with a dataset supporting the effectiveness of gut microbiome interventions in treating PTOA and setting the stage for the first in-human trial work to test targeting of the gut microbiome as an OA disease modifying approach.

- What was accomplished under these goals?

i) Experiments have been initiated to study the gut microbiome dysbiosis that occurs in PTOA (Objective #1). Using a mouse model of PTOA that involves surgical destabilization of the medial meniscus (DMM) to initiate PTOA, we have been collecting fecal samples weekly for 16s rDNA analysis, for study of the metagenome and for study of the metabolome. DMM surgeries were initially performed on a cohort of mice in January of 2020, but these samples were destroyed when the COVID-19 pandemic caused CU and UR to shut down operations in March of 2020. Mice, which were in mid-protocol, were euthanized as a result of the shutdown, essentially rendering collected samples unusable since the full experiment could not be performed. Once the Universities re-opened in the summer of 2020, a new cohort of mice was purchased, and DMMs were performed again in September of 2020. Collection protocols are playing out (CU), and terminal endpoints in December 2020 and January of 2021 will provide fecal and cecal samples to be analyzed and utilized for isolation of various taxa (UR) and then employed in FMT experiments planned in the first half of 2021.

ii) To support humanization FMT experiments (Objective #1), an IRB was developed within the MVM-CoRE to collect fecal material from Veterans with diagnosed advanced knee osteoarthritis that are otherwise healthy. This IRB was originally approved in February of 2020, leading to the planned initiation to obtain HRPO approval. The COVID-19 shutdown in March of 2020 prevented the submission of the HRPO protocol because all human study was suspended, and it was clear a modified protocol would need to be developed with consideration around protections for human participants in the context of the pandemic. Human research restarted in August of 2020 with a broad range of new requirements for COVID-19 protections, and we proceeded to make modification to our human protocol accordingly. The modifications were submitted in September of 2020, and we are waiting for final approval so we can proceed with HRPO review and approval. Our aim is to collect (CU) and analyze (UR) collected fecal samples in the first half of 2021, and then and employ these samples in mouse FMT experiments in the second half of 2021.

iii) We initiated work with hHC supplements immediately after receiving IACUC and ACURO approval in September of 2019 (Objective #2). This work has led to a dataset that was presented at the 2020 American Society for Bone and Mineral Research virtual conference (see appendix 1 for the poster) and is currently in preparation as a manuscript to be submitted to Arthritis Research and Therapy this fall (2020, see appendix 2 for the current draft of the draft figures of the manuscript). This study, which involved quantifying the impact of hHC on PTOA (analysis at CU), provides the first data delineating the gut microbiome impact of this supplement (analysis at UR), setting the stage for the next studies which involve isolation and expansion of several interesting taxa for in vivo work as a probiotic intervention (analysis and both CU and UR). The net results of this study were collected in the broader context of our work to study the role of the gut microbiome in the context of several other nutraceutical products that set the stage for a review article that was published earlier this year and that acknowledges support from this award (see appendix 3).

- What opportunities for training and professional development have the project provided?

We have three trainees that are involved in this project: predoctoral student David Villani and postdoctoral fellows Honey Hendesi MD PhD and Andrew Wu MD (Andrew is supported by other resources independent of this award). We also have technical-level staff (Jake Guzzetti) and junior faculty (Lacey Favazzo PhD) that help carry forward the work. These individuals are involved in all aspects of the management and execution of the experimental plan, and thus gain knowledge on how to administer a program of research, how to consider budgets in the context of the work, and scientific aspects --- the study of PTOA and the gut microbiome. The training and professional development plays out in daily work on the project, weekly work in progress meetings and journal clubs, and ultimately in the presentation of the work in broader contexts (e.g. the American Society of Bone and Mineral Research conference mentioned above. Education is an important component to the

overall plan, and our work on this project dovetails with the broader plan to develop a training program in skeletal biology that will compete for a T32 in 2021.

- How were the results disseminated to communities of interest?

Nothing to Report

- What do you plan to do in the next reporting period to accomplish the goals?

By the end of the next reporting period, we will have completed all experiments in Objective #1 and will be waiting for final results on the humanization FMT experiments. We will also be finishing Objective 2 and setting the stage for initiation of experiments in Objective 3, which will commence in September of 2021. The team is intact, and now fully working collaboratively, so we are confident that we will recover from setbacks related to the COVID-19 shutdown that occurred in 2020.

4. Impact

- What was the impact on development of the principle disciplines of the project?

The central impact of the work so far: Data generated on this project has provided the first evidence suggesting that nutraceutical supplements may impact joint health and OA degenerative disease via effects on the gut microbiome (see appendix 3). This is a novel concept that provides the first explanation for the purported effects of such agents in the context of joint homeostasis and disease.

- What was the impact on other disciplines?

Nothing to report

- What was the impact on technology transfer?

Nothing to report

- What was the impact on society beyond science and technology?

Nothing to report

5. Changes/Problems

- Changes in approach and reasons for change.

Nothing to report

- Actual or anticipated problems or delays and action plans to resolve them

As mentioned, the COVID-19 pandemic has impacted our progress on the project. Research activity at both CU and UR was completely shut down from mid-March of 2020 to June of 2020, with the months between

June and August only leading to incremental increases in campus occupancy and lab/vivarium activity. Human studies were delayed even further, through to September of 2020, due to concerns about community spread and the lagging development of consensus on mitigation of spread. Our human protocol required amendments to move forward because of new policies and procedures that needed to be incorporated into the plan. Amendments are still under review by the IRB at CU as a precursor step to submitting to HRPO for final approval.

Note: There are no anticipated delays in the project moving forward.

- Changes that have significant impact on expenditures

As mentioned, a mouse experiment that was initiated in late 2019 had to be terminated due to the pandemic. Lost costs included the initial purchase of the mice and the per diems for vivarium housing up until the date the colony was euthanized. Summary expenses: 60 male mice + per diems: $1,774.80.

- Significant changes in use and care of human subjects, vertebrate animals, biohazards, and/or select agents.

Nothing to report

6. Products

- Publications, conference papers and presentations

a. Publications:

Favazzo LJ, Hendesi H, Villani DA, Soniwala S, Dar QA, Schott EM, Gill SR, Zuscik MJ. The gut microbiome-joint connection: implications in osteoarthritis. Curr Opin Rheumatol. 2020 Jan;32(1):92-101. PMID: 31724973; PMCID: PMC6903327.

b. Books or other non-periodical, one-time publications

Nothing to report

c. Other publications, conference papers and presentations:

Wu YH, Landgrave SH, Hendesi H, Favazzo LJ, Villani DA, Schroeder W, Thomas SM, Payne KA, Prawitt J, Gill SR, Zuscik MJ. Dietary supplementation with hydrolyzed hyaline cartilage mitigates posttraumatic osteoarthritis: Potential role of shifts in the gut microbiome. American Society for Bone and Mineral Research annual meeting, September 11-15, 2020.

Zuscik MJ. From Gut to OA. Osteoarthritis Research Society International, OARSI Hour entitled “The gut microbiome and OA”. Virtual Live Presentation, August 19, 2020. (This award was acknowledged)

- Website(s) or other internet site(s)

Nothing to report

- Technologies or techniques

Nothing to report

- Inventions, patent applications, and/or licenses

Nothing to report

- Other products

Nothing to report

7. Participants & Other Collaborating Organizations

- What individuals have worked on the project?

University of Colorado School of Medicine (Zuscik Partnering PI)

Name:	Michael Zuscik PhD
Project role:	Partnering PI
Researcher identifier:	
Nearest person month worked:	3
Contribution to project:	Contribution to design and planning of all aspects of the project
Funding support:	

Name:	Lisa Brenner PhD
Project role:	Co-I
Researcher identifier:	Not available
Nearest person month worked:	0.5
Contribution to project:	IRB development and planning for fecal collection from humans
Funding support:	

Name:	Honey Hendesi MD PhD
Project role:	Post-doc
Researcher identifier:	Not available
Nearest person month worked:	6
Contribution to project:	Contribution to design, planning and execution of all aspects of the project
Funding support:	

Name:	David Villani
Project role:	Graduate student

Researcher identifier:	Not available
Nearest person month worked:	12
Contribution to project:	Contribution to design, planning and execution of all aspects of the project
Funding support:	

Name:	Kelly Stearns-Yoder
Project role:	Clinical Coordinator
Researcher identifier:	Not available
Nearest person month worked:	0.6
Contribution to project:	IRB development
Funding support:	

Name:	Jake Guzzetti
Project role:	Clinical Coordinator
Researcher identifier:	Not available
Nearest person month worked:	1.8
Contribution to project:	IRB development
Funding support:	

University of Rochester School of Medicine and Dentistry (Gill Partnering PI)

Name:	Steven Gill PhD
Project role:	Partnering PI
Researcher identifier:	
Nearest person month worked:	3
Contribution to project:	Contribution to design, planning and execution of all aspects of the project, particularly as related to microbiome analysis and microbiology work.
Funding support:	

Name:	Ann Gill MS
Project role:	Senior technical associate
Researcher identifier:	Not available
Nearest person month worked:	6
Contribution to project:	Contribution to microbiome analysis and microbiology work.
Funding support:	

Name:	Cal Palumbo MS
Project role:	Bioinformatician and Data Analyst
Researcher identifier:	Not available
Nearest person month worked:	2.4
Contribution to project:	Contribution to microbiome analysis.
Funding support:	

- Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to report

- What other organizations were involved as partners?

a. Organization Name:
Rousselot BVBA

b. Location of Organization:
Gent, Belgium

c. Partner's Contribution to the Project:
Other: Study material, hydrolyzed hyaline cartilage

8. Special Reporting Requirements

- Collaborative Award

This is a collaborative award. Partnering PIs Michael Zuscik PhD and Steven Gill PhD are located at the University of Colorado and University of Rochester, respectively. This report has denoted which aspects of the project have been completed at each site, so both partnering PIs will be submitting this same document.

- Quad Charts

Not applicable

9. Appendices

Appendix 1: Wu et al, ASBMR poster (2020)
Appendix 2: Figures from draft manuscript to be submitted to Arthritis Research and Therapy
Appendix 3: Favazzo et al, Current Opinion in Rheumatology (2020)

These appendices follow on subsequent pages.

ASBMR 2020 Annual Meeting
September 11–15, 2020
Virtual Event

Dietary Supplementation with Hydrolyzed Hyaline Cartilage Mitigates Posttraumatic Osteoarthritis: Potential Role of Shifts in Gut Microbiome

Y.H. Andrew Wu[1], Samantha H. Landgrave[1,2], Honey Hendesi[1], Lacey J. Favazzo[1], David A. Villani[1,2], William Schroeder[1], Stacey M. Thomas[1], Karin A. Payne[1,2], Janne Prawitt[3], Steven R. Gill[4], Michael J. Zuscik[1,2]

1. Department of Orthopedics, Anschutz Medical Campus, Aurora, Colorado
2. Cell Biology, Stems Cells and Development Program, Anschutz Medical Campus, University of Colorado, Aurora, Colorado
3. Rousselot BVBA, Gent, Belgium
4. Department of Microbiology and Immunology, University of Rochester Medical Center, Rochester, NY

University of Colorado Anschutz Medical Campus

INTRODUCTION

Osteoarthritis (OA) is a leading cause of disability globally. Symptom palliation is the only option for treatment as there is no available disease-modifying therapy. It has recently been suggested that changes in the gut microbiome dysbiosis can influence OA progression ; in fact, the chondroprotective effects of various nutraceuticals, including dietary supplements comprised of cartilage components, may be due to their ability to shift the gut microbiome composition[1,2].

AIM

One supplement in particular, known as hydrolyzed hyaline cartilage (hHC), has anecdotally been identified as joint protective. However, the exact mechanism of this protection and the potential involvement of the gut microbiome is yet to be explored. This study aimed to investigate the basis for joint protection conferred by hHC in OA.

METHOD

- Posttraumatic OA (PTOA) was surgically induced via destabilization of the medial meniscus in male C57BL/6J mice consuming a defined diet from OpenSource (D12451H)
- Injured mice were provided a daily oral supplement of hHC (0.62mg/gm body weight) or vehicle, beginning 2 weeks prior to injury
- Three- and 12-weeks post-injury, knee joints were harvested, fixed, embedded in paraffin and cut sections were stained with Safranin O
- Histomorphometry analyses were performed to measure the area of femur and tibial cartilages
- TNF immunohistochemistry was performed to study joint inflammation and TUNEL staining was performed to assess chondrocyte apoptosis
- Fecal material was also collected to support analysis of the gut microbiome via 16S rDNA sequencing

RESULTS

Histomorphometry revealed that hHC-supplemented mice had more tibial and femoral uncalcified cartilage at both 3- and 12-week post-injury (Figure 1a and b). These results may be related to suppression of inflammation in the hHC cohort, which displayed a trend toward reduced synovial TNF (Figure 2). This paralleled a reduction in chondrocyte apoptosis in hHC-supplemented mice (Figure 3). Suggesting a potential mechanistic association, hHC supplemented mice displayed significant shifts in the gut microbiome that included loss of proinflammatory Peptococcaceae family members, particularly the species rc4-4 (Figure 4).

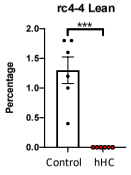

Figure 4: hHC supplemented mice displayed significant shifts in the gut microbiome that included loss of the proinflammatory species rc4-4

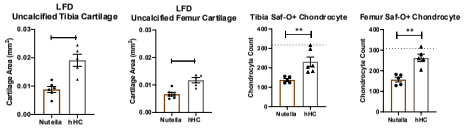

Figure 1a: Cartilage area was evaluated using Osteomeasure System to determine the tibial /femoral uncalcified cartilage area and the total Safranin O-positive chondroctyes in the tibial and femoral cartilage at 12 weeks post-DMM

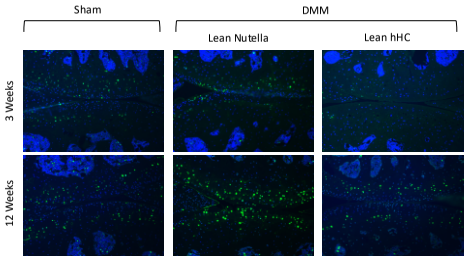

Figure 3: 200x Sagittal sections from the medial compartment of sham and DMM joints 3 weeks and 12 weeks post-DMM were prepared and apoptotic nuclei (green) were identified via TUNE: staining.

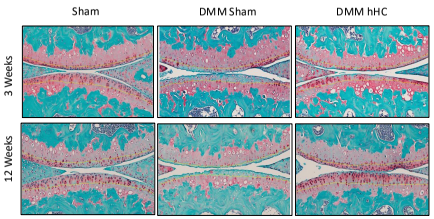

Figure 1b: Array of representative 200x Safranin O/Fast Green stained sagittal sections from the medial compartment of sham and DMM joints 3 weeks and 12 weeks post-DMM

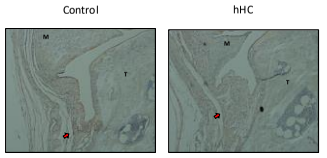

Figure 2: Array of representative 200x TNF immunohistochemistry study, focusing at the synovial located at the junction between tibial plateau (T) and the meniscus (M). Red arrows represent TNF expression positivity

CONCLUSIONS

Findings suggest that oral dietary supplementation with hHC confers joint protective effects in PTOA as well as parallel alterations in the gut microbiome. This sets the stage for follow-on study of the potential mechanistic link between the microbial shifts induced by hHC and its ability to support chondroprotection.

REFERENCES

1. Schott EM, Farnsworth CW, Grier A, Lillis JA, Soniwala S, Dadourian GH, et al. Targeting the gut microbiome to treat the osteoarthritis of obesity. JCI Insight. 2018;3(8).

2. Favazzo LJ, Hendesi H, Villani DA, Soniwala S, Dar QA, Schott EM, et al. The gut microbiome-joint connection: implications in osteoarthritis. Curr Opin Rheumatol. 2020;32(1):92-101.

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DOD IIRA W81XWH1910807; Rousselot BVBA

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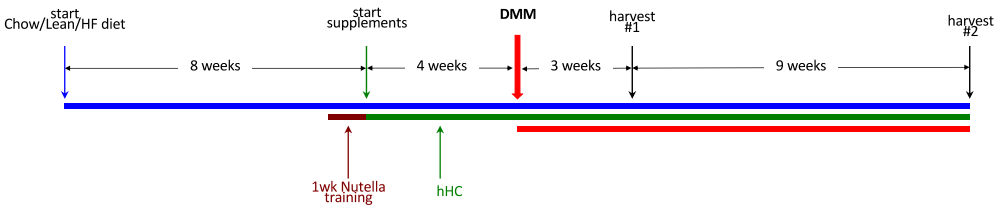
Professor Michael Zuscik
Email: Micheal.Zuscik@cuanschutz.edu

Appendix 2: Figures from draft manuscript to be submitted to Arthritis Research and Therapy

Experimental Timeline



- Six-week old male C57BL/6J mice were placed on standard chow, or lean (10% of calories from saturated animal fat) or HF diet (60% of calories from saturated animal fat)
- Destabilization of the medial meniscus (DMM): Model to induce posttraumatic OA
- Harvest tissue joint, blood, and fecal material after 3 and 12 weeks on supplements

Experimental Timeline

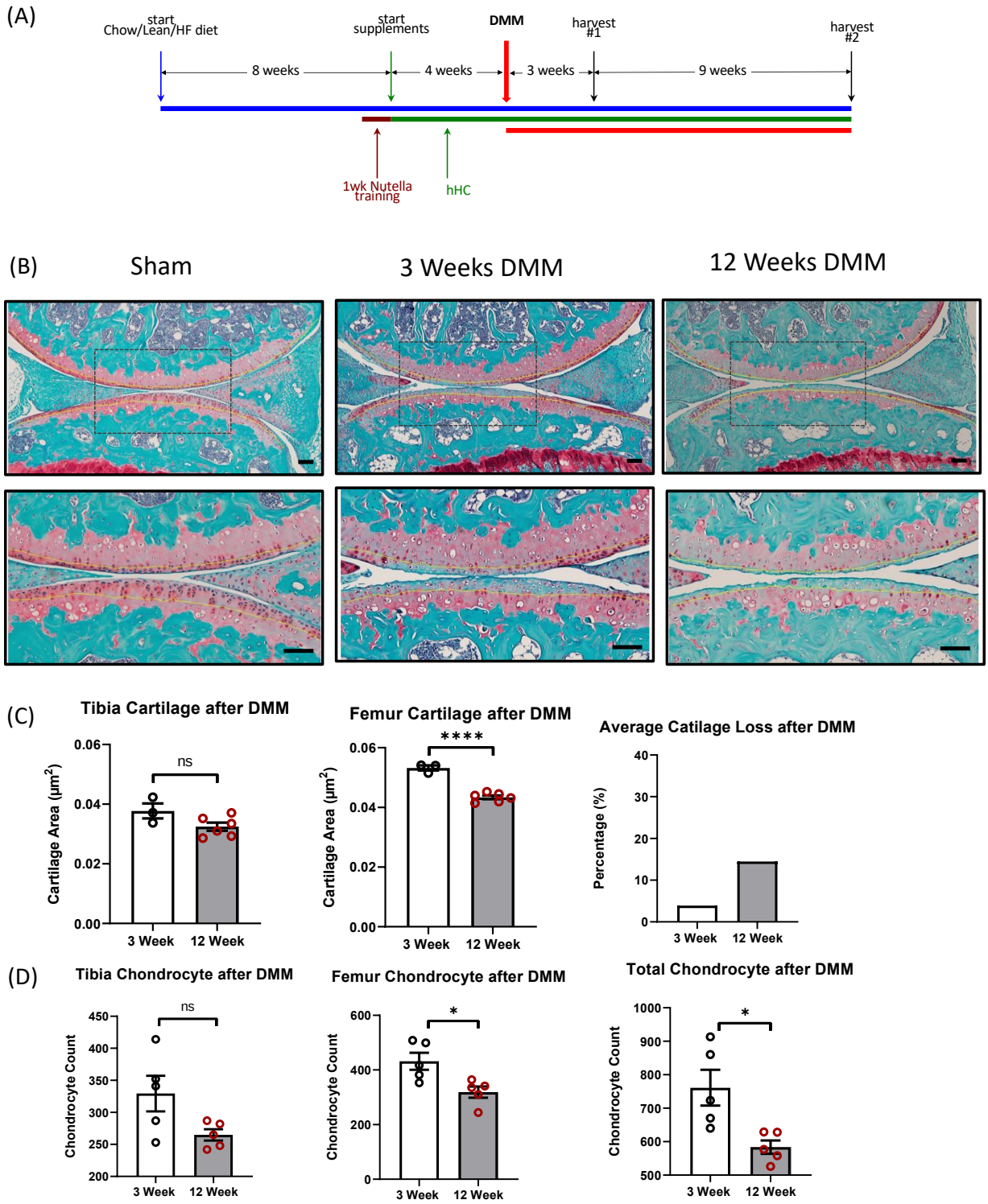
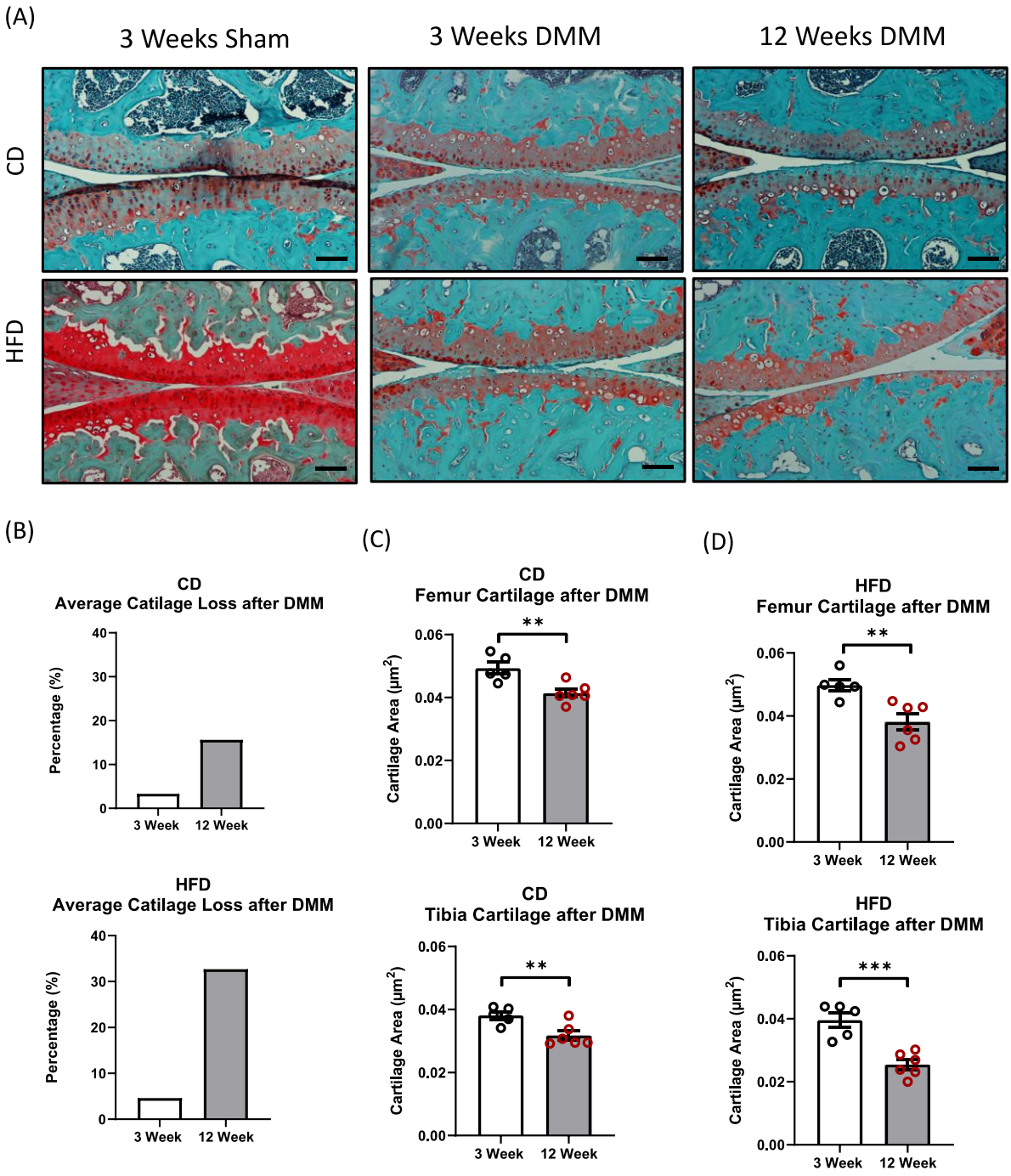


Figure 1: Impact of DMM injury on OA progression at 3 and 12 weeks post-injury in mice fed the lean diet.

Supplementary



Supplemental Figure 1: Impact of DMM injury on OA progression at 3 and 12 weeks post-injury in mice fed either a chow or high fat diet.

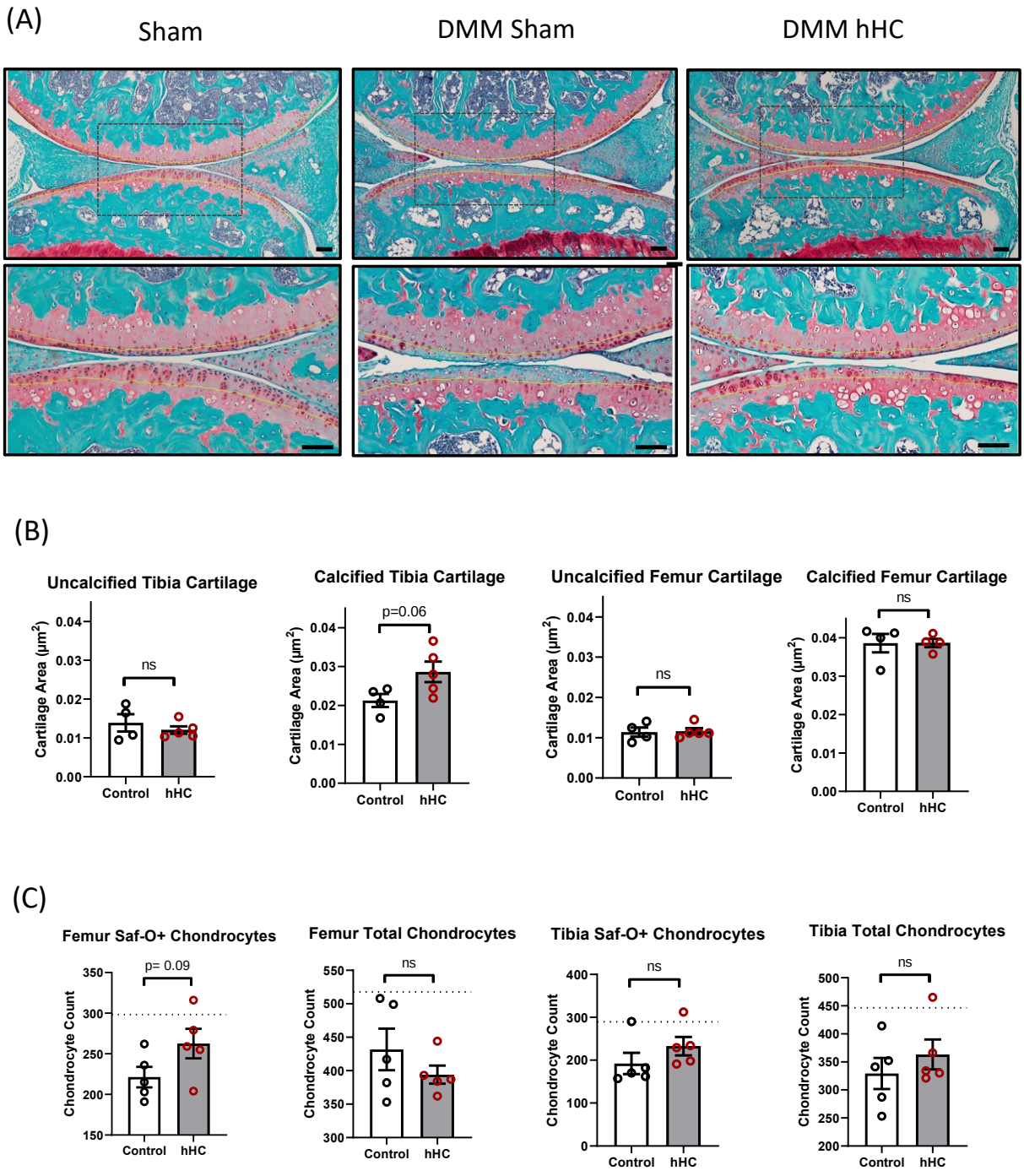
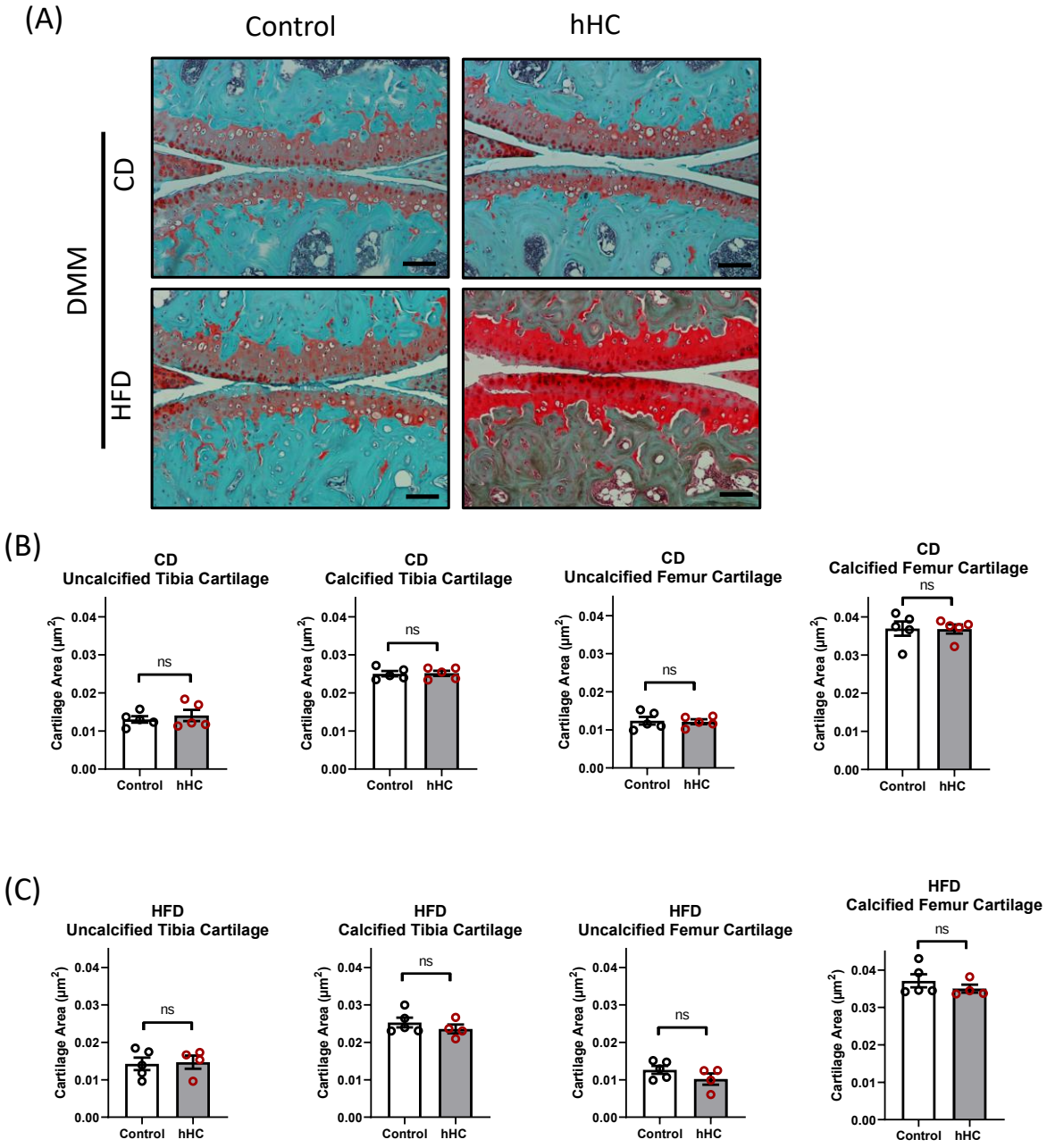


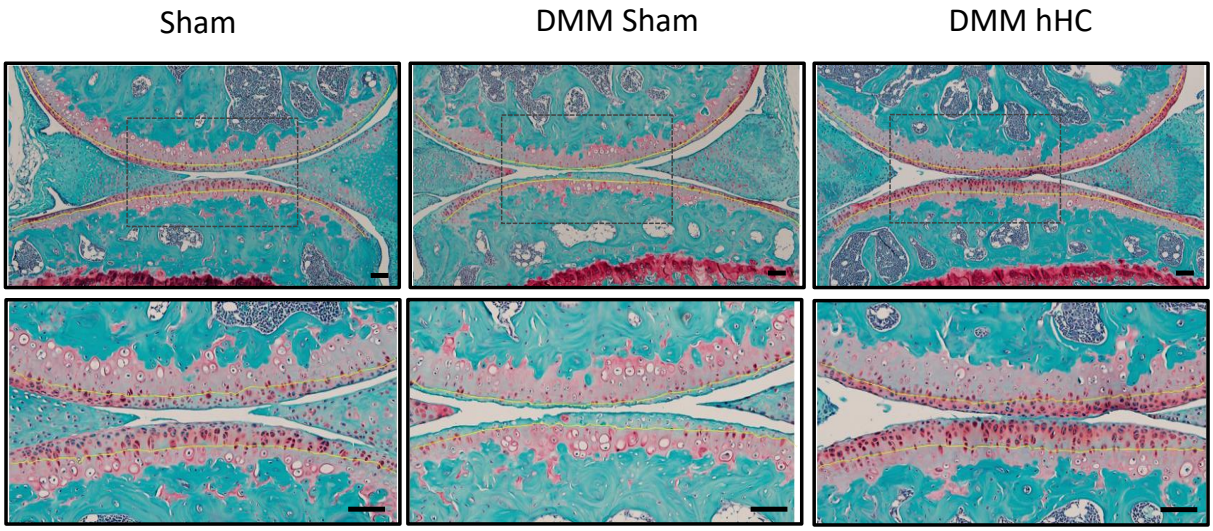
Figure 2: Impact of daily hHC supplementation on PTOA at 3 weeks post injury in mice fed the lean diet.

Supplementary

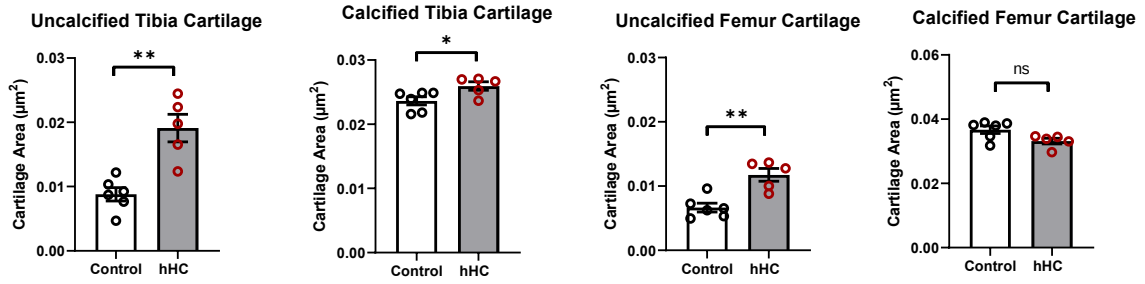


Supplemental Figure 2: Impact of daily hHC supplementation on PTOA at 3 weeks post injury in mice fed either chow or high fat diet.

(A)



(B)



(C)

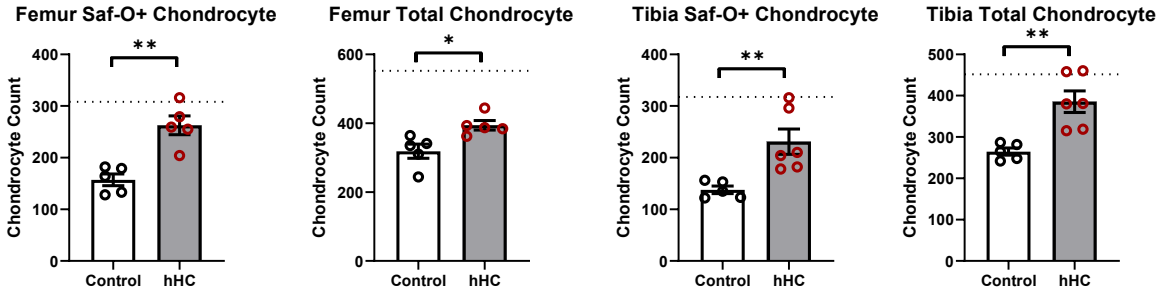
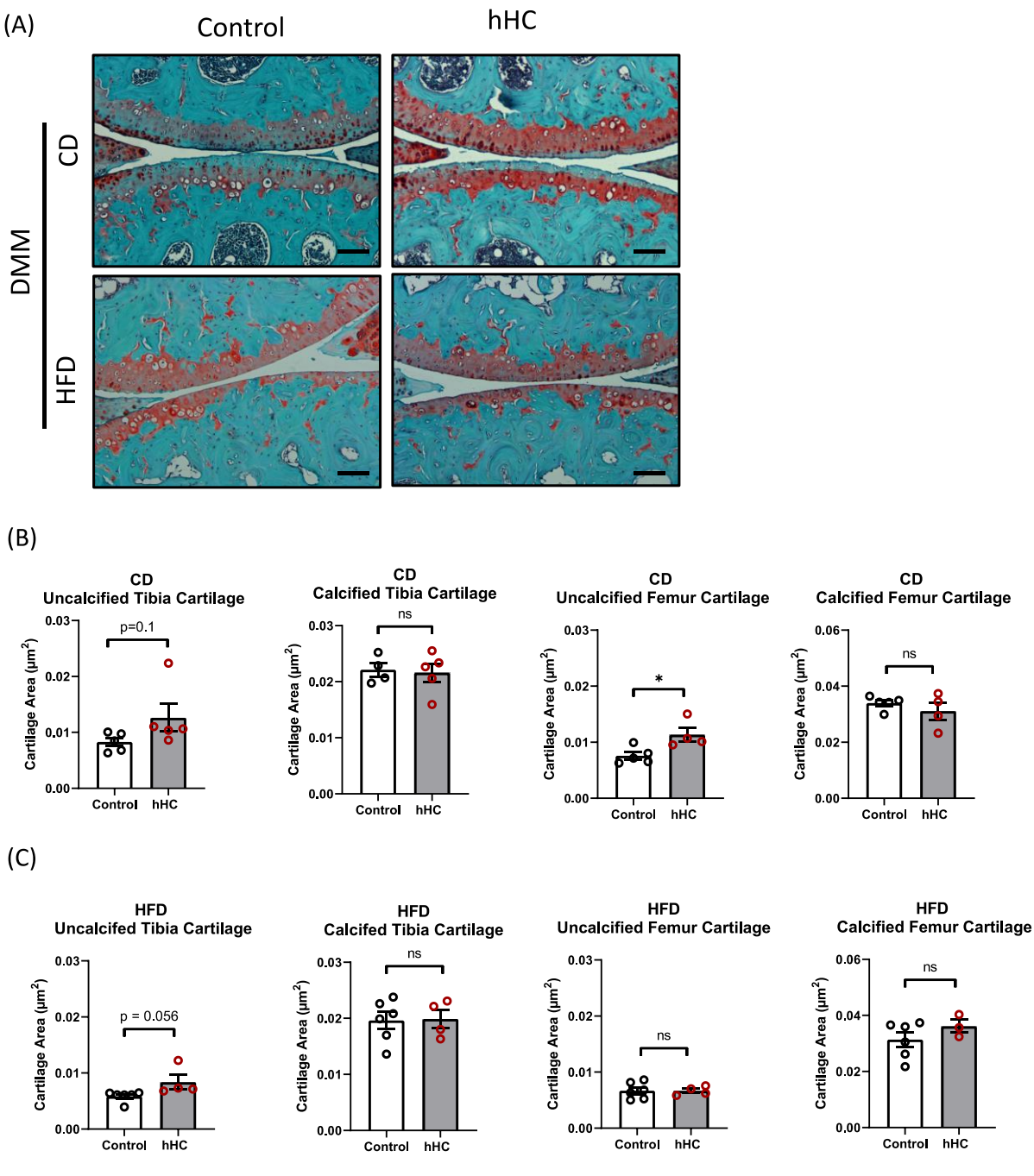


Figure 3: Impact of daily hHC supplementation on PTOA at 12 weeks post injury in mice fed the lean diet.

Supplementary



Supplemental Figure 3: Impact of daily hHC supplementation on PTOA at 3 weeks post injury in mice fed either chow or high fat diet.

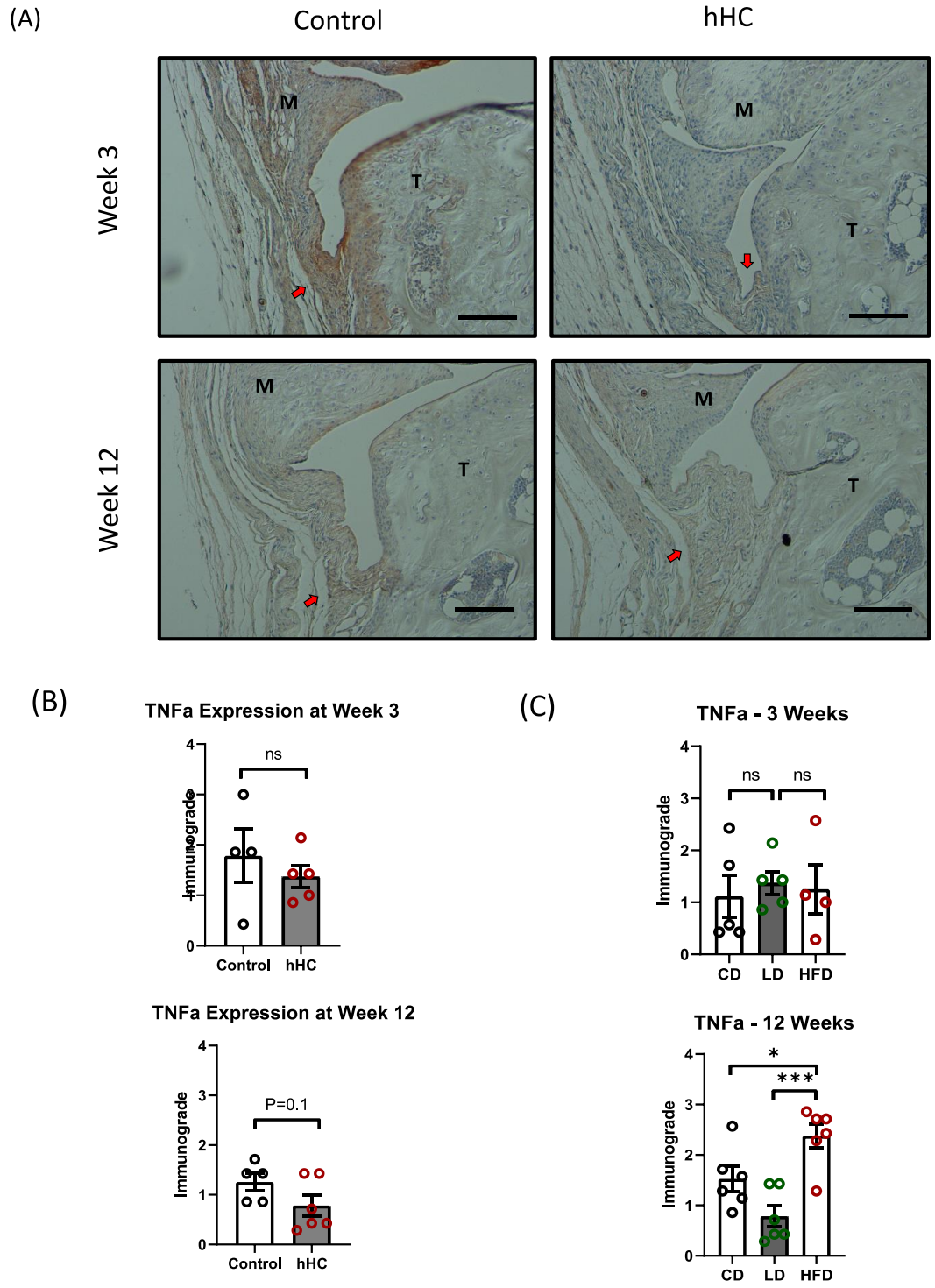
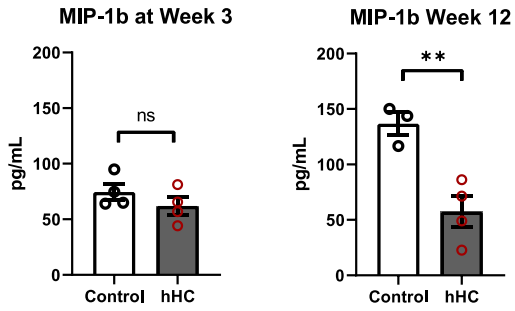


Figure 4: Impact of daily hHC supplementation on TNF levels in the synovium of mice administered the DMM injury and fed the lean diet.

Supplementary

(A)



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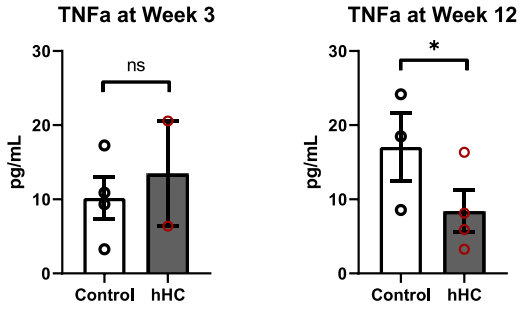

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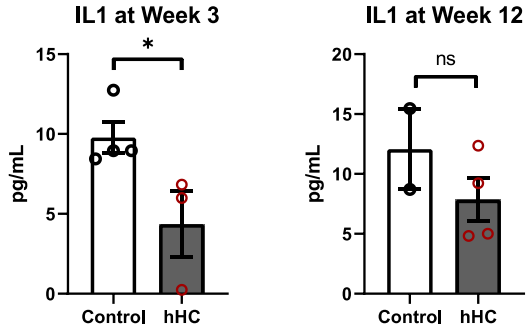

Supplemental Figure 4: Impact of daily hHC supplementation on circulating cytokines in DMM injured mice fed the high fat diet.

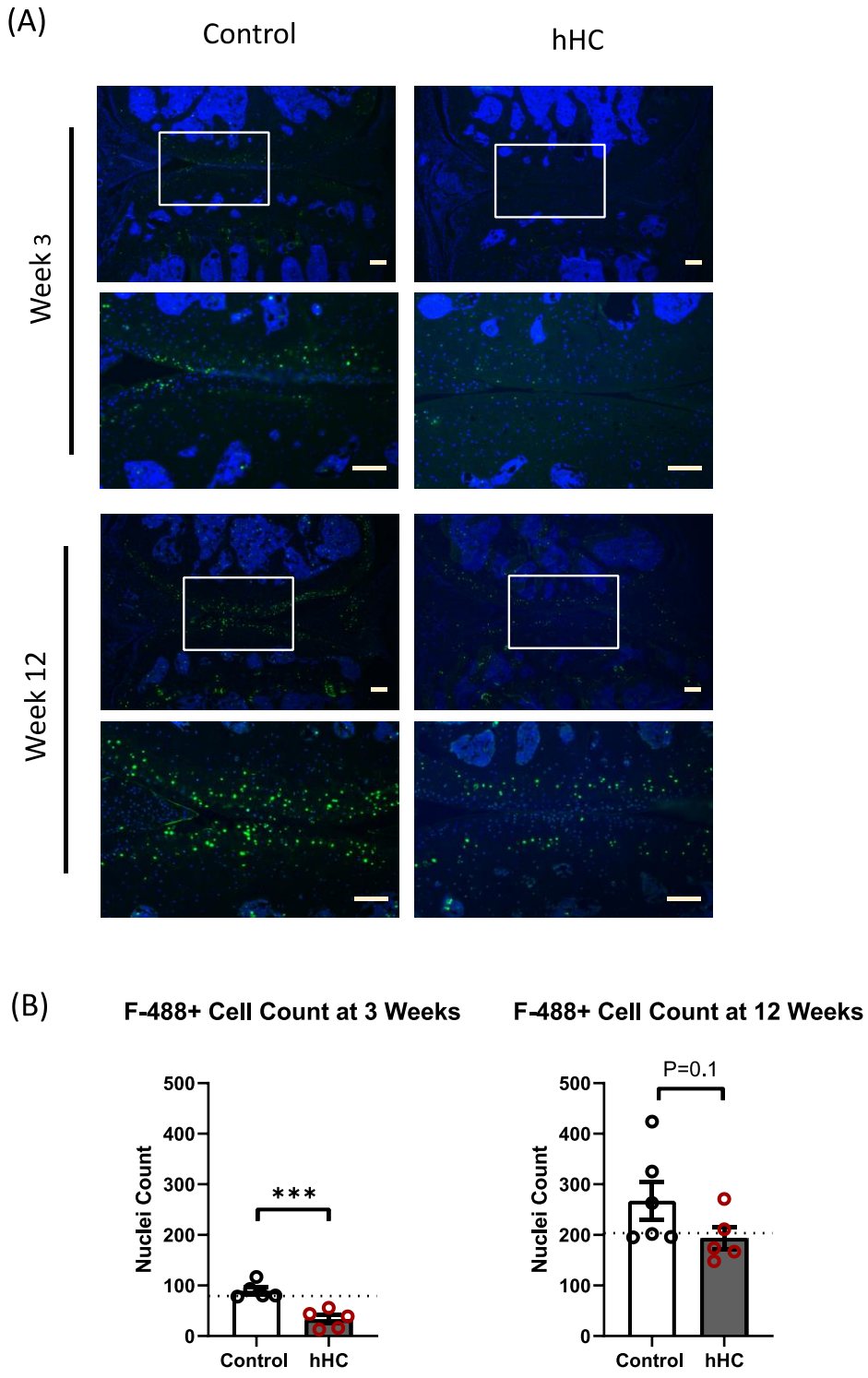

Figure 5: Impact of daily hHC supplementation on chondrocyte apoptosis in DMM injured mice fed the lean fat diet at 3 and 12 weeks post-injury.

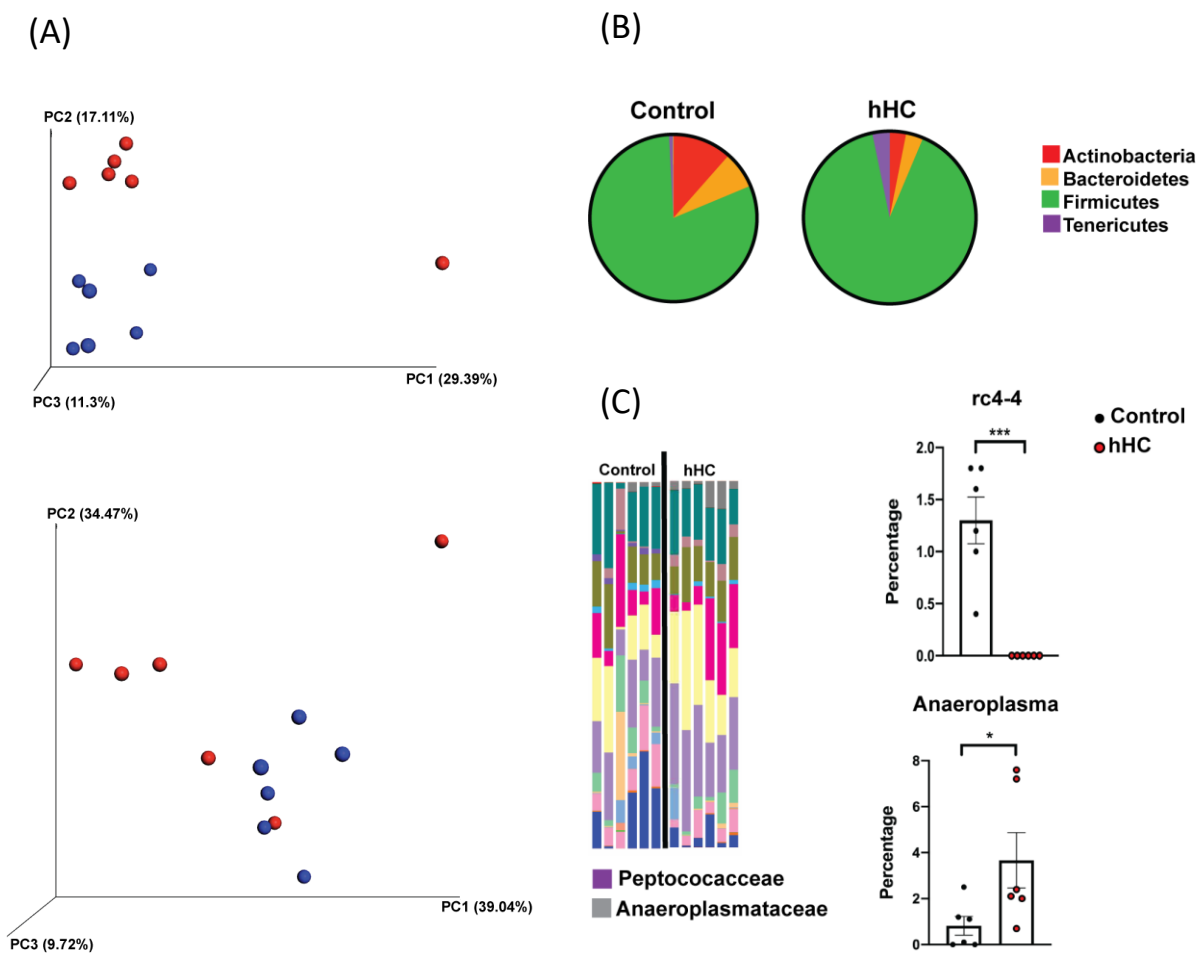

Figure 6: Daily dietary supplementation of lean fed mice with hHC leads to significant shifts in the gut microbiome.

CURRENT OPINION

The gut microbiome-joint connection: implications in osteoarthritis

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Purpose of review

Osteoarthritis is a debilitating disease leading to joint degeneration, inflammation, pain, and disability. Despite efforts to develop a disease modifying treatment, the only accepted and available clinical approaches involve palliation. Although many factors contribute to the development of osteoarthritis, the gut microbiome has recently emerged as an important pathogenic factor in osteoarthritis initiation and progression. This review examines the literature to date regarding the link between the gut microbiome and osteoarthritis.

Recent findings

Studies showing correlations between serum levels of bacterial metabolites and joint degeneration were the first links connecting a dysbiosis of the gut microbiome with osteoarthritis. Further investigations have demonstrated that microbial community shifts induced by antibiotics, a germ-free environment or high-fat are important underlying factors in joint homeostasis and osteoarthritis. It follows that strategies to manipulate the microbiome have demonstrated efficacy in mitigating joint degeneration in osteoarthritis. Moreover, we have observed that dietary supplementation with nutraceuticals that are joint protective may exert their influence via shifts in the gut microbiome.

Summary

Although role of the microbiome in osteoarthritis is an area of intense study, no clear mechanism of action has been determined. Increased understanding of how the two factors interact may provide mechanistic insight into osteoarthritis and lead to disease modifying treatments.

Keywords

glucosamine, gut microbiome, osteoarthritis, undenatured type 2 collagen

INTRODUCTION

Osteoarthritis is a multifaceted whole joint disease involving degeneration of articular cartilage, subchondral bone sclerosis and synovial inflammation with these combine symptoms culminating in joint pain and disability [1]. Impacting more than 10% of the US population, osteoarthritis poses an enormous economic burden comprised of medical care costs, lost wages, and reduced economic productivity, with significant impact on the quality-of-life of its sufferers [2,3]. Despite its clinical and financial ramifications, there are currently no approved disease modifying osteoarthritis drugs available, with symptom palliation the only alternative [4]. Osteoarthritis is now recognized as a disease of complex cause, including age, injury, genetics, sex, and obesity as central contributing factors [5]. A commonality shared by many of these contributors is chronic systemic inflammation [6–11], and emerging

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KEY POINTS

- Dysbiosis contributes to the development of osteoarthritis.
- Shifts in gut microbiome composition may reduce progression of osteoarthritis.
- The microbiome is an emerging target for osteoarthritis therapeutics.
- Joint protective nutraceuticals may act by shifting the gut microbiome.

research has firmly linking the inflammation of obesity and osteoarthritis initiation and progression [11,12,13▪▪].

The microbiome is the totality of the microbial ecosystems that exist within and on the human body, including both organisms and their secreted products [14–16]. The microbiome is a crucial component of the holobiont, and has significant ramifications for human health [17]. A growing understanding of the gut microbiome ascribes to it both endocrine and immunological function [18,19]. The number of bacterial cells in the human microbiome likely exceeds the number of host cells, with this ratio ranging anywhere from 1 : 1 up to estimates that bacterial cells outnumber human cells by a factor of 100 [20,21]. Wet mass of the microbiome has been calculated to equal that of the human kidney, supporting the notion that it could fundamentally serve an endocrine-like regulatory role.

Defining a healthy microbiome is a difficult endeavor owing to the large variations in what could be considered 'normal' caused by diurnal rhythms, immune status, diet, genetics, and many other variables [22–26]. However, shifts in the microbiome have an established role in numerous diseases, including amyotrophic lateral sclerosis, Parkinson's disease, Alzheimer's disease, rheumatoid arthritis (RA), Crohn's disease, type 2 diabetes, metabolic syndrome, and osteoporosis [27–32,33▪▪]. A common factor in many of these diseases is chronic local and systemic inflammation, and it is now understood that the gut microbiome contributes to inflammation by inducing the production of proinflammatory cytokines by host immune cells and by production of inflammatory bacterial metabolites [34]. Given that osteoarthritis is now understood to involve an inflammatory component, particular in the context of obesity [35,36], and as it is established that the gut microbiome is a regulator of inflammation [37–39], studies investigating the connections between the gut microbiome and the degenerative processes of osteoarthritis are crucial. In this review we will provide a state of the field assessment on work connecting the gut, joint, and osteoarthritis.

Most of our knowledge about the role of the gut microbiome in the development of inflammation-driven diseases has been generated outside the context of the skeletal system. Recently, however, a number of studies have provided data suggesting a role for the gut microbiome in bone homeostasis, RA, and most recently in osteoarthritis initiation and progression. In the context of osteoarthritis, these studies have used various rodent models of joint degenerative disease such as high-fat diet (HFD)-induced obesity, mechanical over-loading, surgical induction, and genetically prone rodent models; in all cases potential contributions of the gut microbiota to progression of osteoarthritis have been suggested. Table 1 provides a summary of the key published works that contribute to the current state of the field. Hinting at human relevance, a couple studies have suggested that patients diagnosed with osteoarthritis possess a quantifiable dysbiosis in the gut microbiome, supporting the concept that there is an osteoarthritis-associated microbial shift that may be pathogenic. As obesity and metabolic syndrome are known to be caused by gut microbiome dysbiosis and are important risk factors in the development of osteoarthritis [6,40], it follows that among all causes of osteoarthritis, potential causation in this context has been the most extensively investigated. Studies have shown that human obesity is associated with increased osteoarthritis risk in both weight bearing and nonweight bearing joints [41], suggesting the possibility of systemic players in the development of the osteoarthritis of obesity. In addition, studies on obesity in conjunction with other causes of osteoarthritis, such as injury [destabilization of the medial meniscus (DMM) or intra-articular fracture surgery] have reported an enhanced osteoarthritis severity, thus suggested a synergic effect of obesity [41,42].

STUDIES CONNECTING GUT MICROBIOME AND OSTEOARTHRITIS

Study of a potential gut microbiome-joint axis in homeostasis and disease is a nascent area of study. Early work involved rodent models of osteoarthritis and in humans diagnosed with osteoarthritis, revealing a correlation between increased levels of circulatory inflammatory markers including bacterially produced lipopolysaccharides (LPS) that correlate with osteoarthritis severity, suggesting that microbiome-derived proinflammatory metabolites are players in osteoarthritis [43,44]. In a study of rats fed high-fat/high-sugar diet for 28 weeks, Collins *et al.* showed increased cartilage damage in obese animals and established a direct correlation

Table 1. Literature review summary

Study	Animal model	OA inducing method	Intervention	OA assessment method	Microbiome Assessment	Conclusion
Collins *et al.*, 2015 [43]	Rats (male)	High fat/high sucrose diet for 28 weeks. (40% energy from fat and 45% from sucrose)	N/A	Cartilage histological evaluation (Modified Mankin scores), synovial and sub-chondral bone assessment (OARSI), serum and synovial fluid biomarkers analysis	Fecal sample 16s sequencing	Higher Mankin scores, and higher level of serum LPS in obese animals. *Lactobacillus* species and *Methanobrevibacter spp.* abundance correlated with Mankin scores.
Huang *et al.*, 2016 [44]	Humans (male and female)	N/A	N/A	Knee Radiography, Etarfolatide SPECT/CT scans, serum and synovial fluid biomarker analysis, WOMAC questionnaire, NHANES-I pain scores	N/A	Serum LPS and LBP levels were associated with an increased abundance of activated macrophages in the knee joint capsule and synovium; they were also associated with osteophyte formation, knee joint space narrowing, and WOMAC scores.
Ulici *et al.*, 2018 [45▪]	Germ Free Mice (male)	DMM surgery	N/A	Histologic cartilage evaluation (ACS scores), osteophyte and synovial membrane evaluation	Fecal samples 16s sequencing	Reduced ACS scores in germ free mice compared to the control group. Differences in abundance of microbes detected in high and low ACS mice.
Schott *et al.*, 2018 [13▪▪]	Mice (male)	High fat diet induced obesity (60% kcal from fat for 12 weeks) and DMM surgery	Oligofructose (prebiotic)	Cartilage histological evaluation (OARSI scores), IHC and IF of cartilage and synovium, PAST cells flow cytometry, Micro CT scan of mineralized meniscus, serum biomarkers analysis	Fecal samples 16s sequencing	Prebiotic supplementation reduced OA severity observed in HFD mice. *Bifidobacterium pseudolongum* abundance increased with prebiotic treatment; its levels were inversely related to the OA severity, systemic, and colon inflammation.
Guss *et al.*, 2019 [47]	Mice (male)	Metabolic disorder (TLR5KO), High fat diet induced obesity (60% energy from fat)	Tibial mechanical loading (2 weeks or 6 weeks)	Cartilage histological evaluation (OARSI scores and Modified Mankin scores), Micro CT evaluation of the subchondral bone trabecular architecture, serum biomarker analysis	Fecal samples 16s sequencing	Long term (6 weeks) mechanical loading is necessary to induce OA. Mild metabolic disorder is not sufficient to develop severe OA. Disturbing metabolic disorder with antibiotics reduces OA severity.
Rios *et al.*, 2019 [49▪▪]	Rats (male)	High fat/high sucrose diet for 28 weeks (20% energy from fat, 50% from sucrose)	Prebiotic fibers supplementation and exercise (12 weeks)	Cartilage histological evaluation (Modified Mankin scores), synovial and sub-chondral bone assessment (OARSI), serum and synovial fluid biomarker analysis	Cecal matter 16s sequencing	Prebiotic fibers, exercise, and the combination of both prevented OA development. Prebiotic treatment increased *Bifidobacterium* and *Roseburia;* they decreased *Closteridium leptum* and *Akkemansia muciniphila.*
Henrotin *et al.*, 2019 [50▪]	Dunkin Hartley Guinea pigs (male)	Spontaneous development	Lyophilized inactivated culture from *Bifidobacteri-um longum* CBi0703 (12 weeks)	Cartilage histological evaluation (OARSI scores), serum biomarkers analysis	N/A	*B. longum* oral administration decreased cartilage damage and decreased type II collagen degradation.

Studies investigating the connection between osteoarthritis development and the gut microbiome in rodent models of osteoarthritis are enumerated. Relevant studies were identified through a PubMed search for articles in English published in peer-reviewed journals. We used multiple search terms capturing osteoarthritis, joint, cartilage, gut microbiome and microbiota. Articles selected were reviewed by all coauthors for final inclusion. ACS, articular cartilage structure; CT, computed tomography; HFD, high-fat diet; IF, immunofluorescence; IHC, immunohistochemistry; LBP, LPS binding protein; LPS, lipopolysaccharides; OA, osteoarthritis; SPECT, single-photon emission computerized tomography.

between serum LPS levels and Mankin histological scores. When the gut microbiome composition was examined by 16S sequencing, they detected an increase in *Lactobacillus* spp. and *Methanobrevibacter* spp. abundance with a strong predictive relationship with histological score [43]. Significantly, in gnotobiotic mice, Ulici *et al.* [45▪] showed a decline in the severity of posttraumatic osteoarthritis in the germ-free situation; this provided evidence for a role of the gut microbiome in osteoarthritis pathogenesis in this model. Suggesting relevance in humans, in 25 patients with knee osteoarthritis, Huang *et al.* [44] established a link between serum and synovial fluid LPS levels with activated macrophages in the knee joint capsule and synovium, joint space narrowing, osteophyte formation, and increased WOMAC scores (i.e., worse symptoms). Connecting these LPS-osteoarthritis associations with a possible dysbiosis in the gut microbiome occurred in a study of the Rotterdam cohort. In 1444 participants enrolled in the Rotterdam study-III with hip and/or knee osteoarthritis, an association between increased WOMAC score and abundance of microbes in the proinflammatory *Streptococcus* taxa was identified [46▪]. This study further established human relevance, prompting the field to more carefully examine the role of a dysbiotic community or individual taxa as pathogenic in osteoarthritis.

To investigate the role of metabolic dysfunction in the absence of overt obesity on gut-joint associations and the involvement of individual taxa in this context, Guss *et al.* used a murine genetic model of metabolic syndrome (Toll-like receptor-5 deficiency) in combination with osteoarthritis-inducing mechanical overloading (2 or 6 weeks). The authors compared the impact of the metabolic disorder in this context to previously studied HFD-induced osteoarthritis models. Evaluation of histological changes in the cartilage indicated more severe osteoarthritis in the HFD-fed group; in this group they detected metabolic irregularities, increased body fat, systemic inflammation and the expected gut microbiome dysbiosis which included an increased abundance of Firmicutes. They concluded that while metabolic irregularities were observed in Toll-like receptor-5 deficient mice, alone they were not sufficient to induce osteoarthritis. Rather, they showed that increased levels of LPS in HFD-fed mice was associated with higher OARSI scores and a dysbiosis involving expansion of Firmicutes, suggesting an association between microbial components and development of osteoarthritis [47].

In an article providing evidence that gut microbiome dysbiosis in obesity is more than just associated with osteoarthritis, Schott *et al.* [13▪▪] examined microbial community shifts in HFD-fed obese mice with an overlay of DMM injury to synchronize initiation of disease. Histological evaluation showed increased cartilage degradation in HFD-fed obese mice and 16S sequencing confirmed a gut dysbiosis. Importantly, when HFD-fed obese mice were supplemented with the indigestible fiber oligofructose, the obese gut dysbiosis was mitigated and osteoarthritis progression was essentially halted. *Bifidobacterium pseudolongum*, a species with known anti-inflammatory properties [48] that was lost in HFD-fed mice, was restored following oligofructose supplementation [13▪▪]. Conversely, proinflammatory *Peptococcaceae* and *Peptostreptococcaceae* family members that were present in the obese cohort were completed ablated in oligofructose-supplemented mice. These findings provided the first published evidence that shaping of the microbial community with an indigestible prebiotic fiber, lacking direct effects on host biology, could be disease modifying in osteoarthritis; implication from the work indicate a causal link between gut microbiome dysbiosis and osteoarthritis.

Consistent with the Schott *et al.* findings, HFD-fed rats supplemented with oligofructose also showed delayed development of obesity-associated osteoarthritis [49▪▪]. In this report, Rios *et al.* demonstrated that a maximum protection could be achieved by combining the supplement with exercise. Paralleling the Schott *et al.* study, 16S sequencing demonstrated an increase in *Bifidobacterium* and *Roseburia* and a decrease in *Clostridium leptum* and *Akkermansia muciniphila* levels as a result of oligofructose supplementation [49▪▪]. Another recent study in a guinea pig model of spontaneous osteoarthritis showed that the oral administration of a lyophilized inactivated culture of *Bifidobacterium longum CBi0703* reduces cartilage structural lesions and cartilage degradation markers, providing an overall joint protective effect [50▪]. Future studies on microbes from the *Bifidobacterium* taxa and their metabolites may be important in establishing a gut-joint axis and may represent a subset of new approaches to treat osteoarthritis that involve targeting the gut microbiome.

NUTRACEUTICALS MAY IMPROVE OSTEOARTHRITIS OUTCOMES VIA EFFECTS ON THE GUT MICROBIOME

Glucosamine, chondroitin sulfate, and undenatured type 2 collagen (UT2C) are nutraceuticals marketed as dietary supplements supportive of joint health. In the US alone, these compounds make up a multibillion-dollar industry that spans both human and animal use [51]. In the first major article detailing the protective effects of nutraceuticals on joint disease, Trentham *et al.* [52] demonstrated that oral

consumption of rooster comb type 2 collagen had beneficial effects on biological, structural, and pain outcomes in patients with RA flare. Although this provocative finding was met with broad skepticism because of uncertainty regarding mechanism of action, it was an important early piece of evidence for use of cartilage component-based nutraceuticals to treat joint arthropathies. Various preclinical experiments have also supported the notion that oral supplementation with 'joint protective' nutraceuticals is effective at mitigating osteoarthritis. For example, Dar *et al.* [53] demonstrated that daily

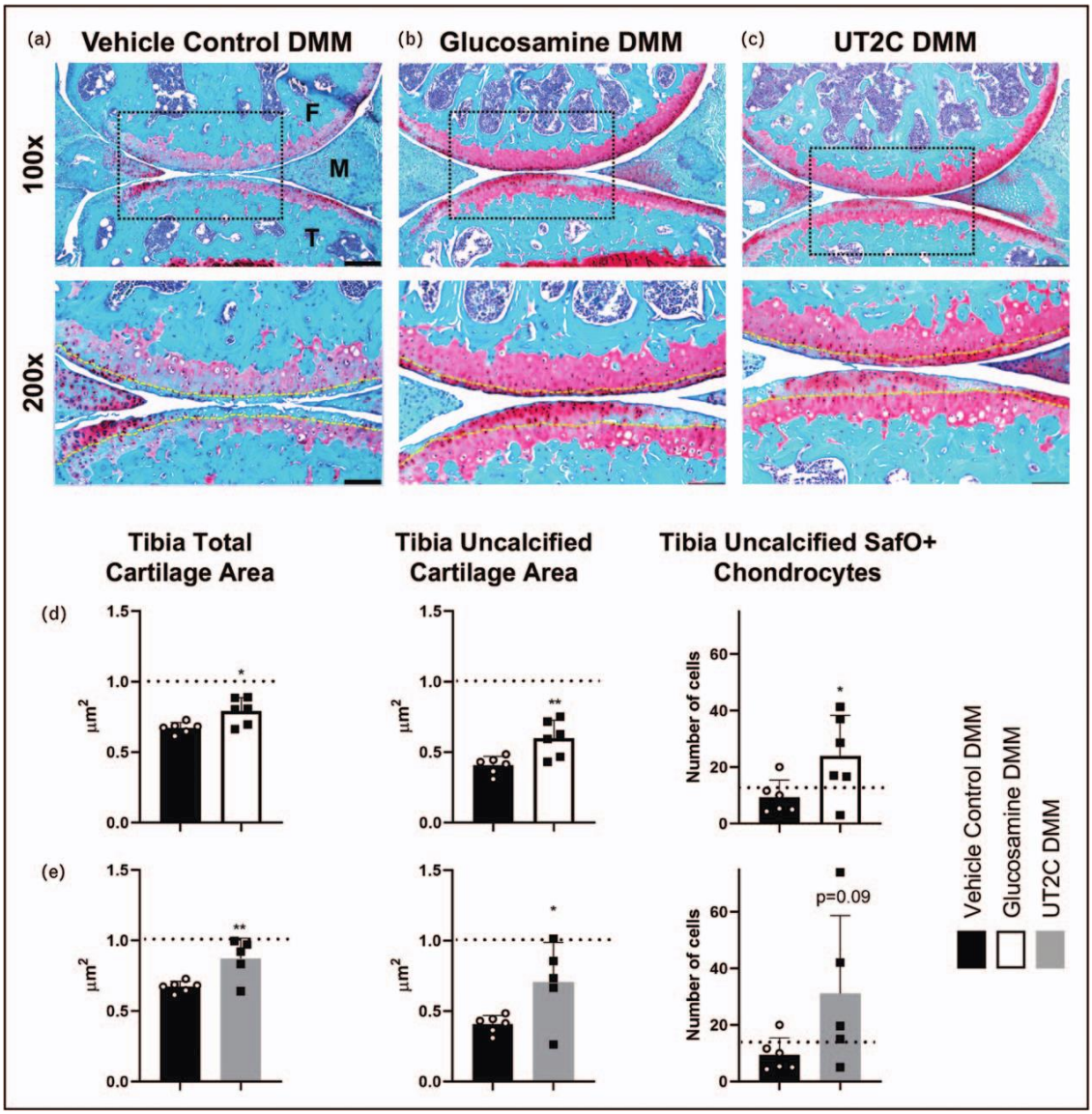

FIGURE 1. Glucosamine and undenatured type II collagen ameliorate posttraumatic osteoarthritis compared with vehicle control. Sham or DMM surgery was initiated on the knee joint 2 weeks after either glucosamine or undenatured type II collagen were introduced in the diet. Representative Safranin O/Fast Green stains collected from mice fed vehicle control (a) glucosamine (b) or undenatured type II collagen (c) are presented (Yellow dotted line = tidemark, Black dotted line box = region of interested viewed at higher magnification, F = femur, M = meniscus, T = tibia, Scale bar at 100× = 100 µm, Scale bar at 200× = 50 µm). Sections like those in (a–c) were used for histomorphometry. Tibia cartilage area, tibia uncalcified cartilage area, and SafO+ chondrocytes were quantified for mice fed glucosamine (d) and undenatured type II collagen (e) compared with vehicle control. Dashed black lines indicate measurement on sham knee joints. Data shown represent mean ($n \geq 5$) ± SD; statistical significance was determined using Student's *t* test $^{*}P < 0.5$, $^{**}P < 0.01$. DMM, destabilization of the medial meniscus.

oral consumption of hydrolyzed type I collagen is chondroprotective in murine post traumatic osteoarthritis. Similarly, Bagi *et al.* [54] demonstrated that oral supplementation with undenatured native chicken type 2 collagen reduced joint degeneration in a rat model of posttraumatic osteoarthritis. As oral supplements composed of cartilage and soft tissue matrix components are the only agents with clinical data supporting positive patient-reported functional improvement in osteoarthritis [55–57], we and others have speculated that positive results may be due to an unappreciated action of these agents as prebiotics that can affect the gut microbiome.

Suggesting a potential mode of biological action that involves shaping of the gut microbiome, several studies have documented microbial shifts is response to chondroitin sulfate [58–60]. In the Liu *et al.* [58] study particularly, supplementation was associated with reductions in inflammatory Proteobacteria and increases in a Bacteroidetes taxa that blocks stress-induced intestinal inflammation. These shifts suggest a potential mode of action in osteoarthritis. To address this possibility, we supplemented mice on a chow-based diet with two popular joint-protective nutraceuticals (see Supplementary data, http://links.lww.com/COR/A48). Mice were supplemented daily with either 0.31 mg/g of body weight of glucosamine or 2 μg/g of body weight of UT2C to examine their joint protective capabilities and to examine changes in the gut microbiome. In chow fed mice, osteoarthritis was initiated by trauma (DMM surgery), and as expected, we observed degenerative change to the cartilage in injured joints compared with sham-operated controls. In mice given the same injury but fed a diet supplemented with either glucosamine or UT2C, we observed a deceleration of degenerative change (Fig. 1a–c), evidenced by a general improvement in OARSI scores of mice supplemented with glucosamine (2.16 ± 0.98) or UT2C (1.88 ± 0.84) compared with the vehicle control (2.54 ± 0.52). Histomorphometric analysis of joint cartilage revealed increased tibia total cartilage area, tibia uncalcified cartilage area, and number of Safranin O^+ chondrocytes all in both glucosamine and UT2C supplemented mice compared with the vehicle control (Fig. 1d and e).

16S sequencing of DNA extracted from fecal pellets harvested from these mice revealed changes in the abundance of Bacteroidetes, Actinobacteria, and Firmicutes, among others (Fig. 2a). Firmicutes and Bacteroidetes are typically the dominant phyla of the vertebrate microbiome [61], and an increase

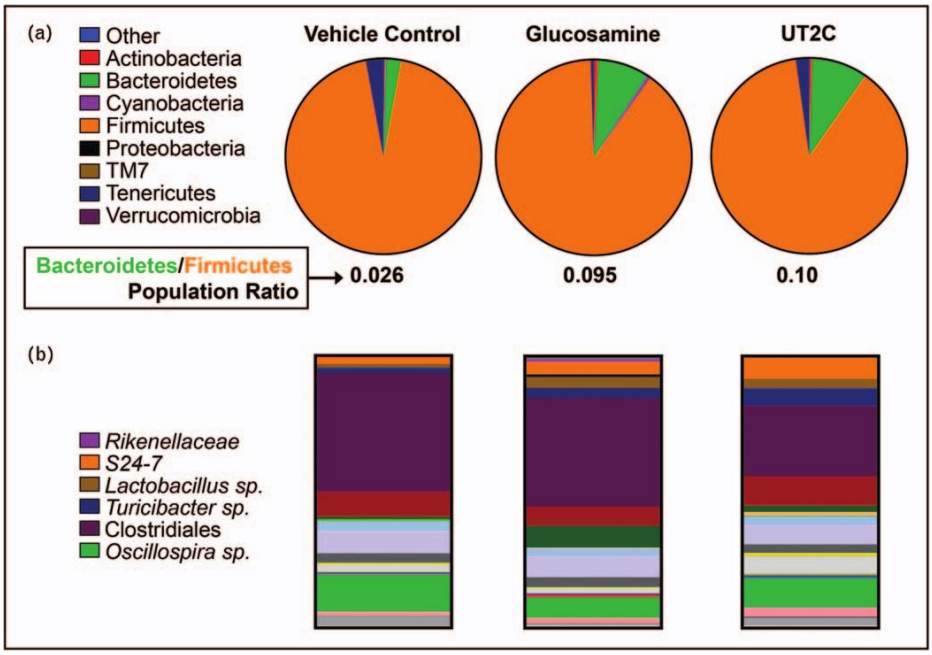

FIGURE 2. Supplementation with glucosamine or undenatured type II collagen impacts the gut microbiome. (a) Phylum level relative abundances change when either glucosamine or undenatured type II collagen are included in the diet for 12 weeks following injury compared with a vehicle control. Data shown are average relative abundances of phyla in each experimental group ($n = 3$). (b) Relative abundance of operational taxonomic unit in the gut microbiome determined by fecal sampling is changed by supplementation with either glucosamine or undenatured type II collagen. Data shown are average relative abundances of operational taxonomic unit in each experimental group ($n = 3$).

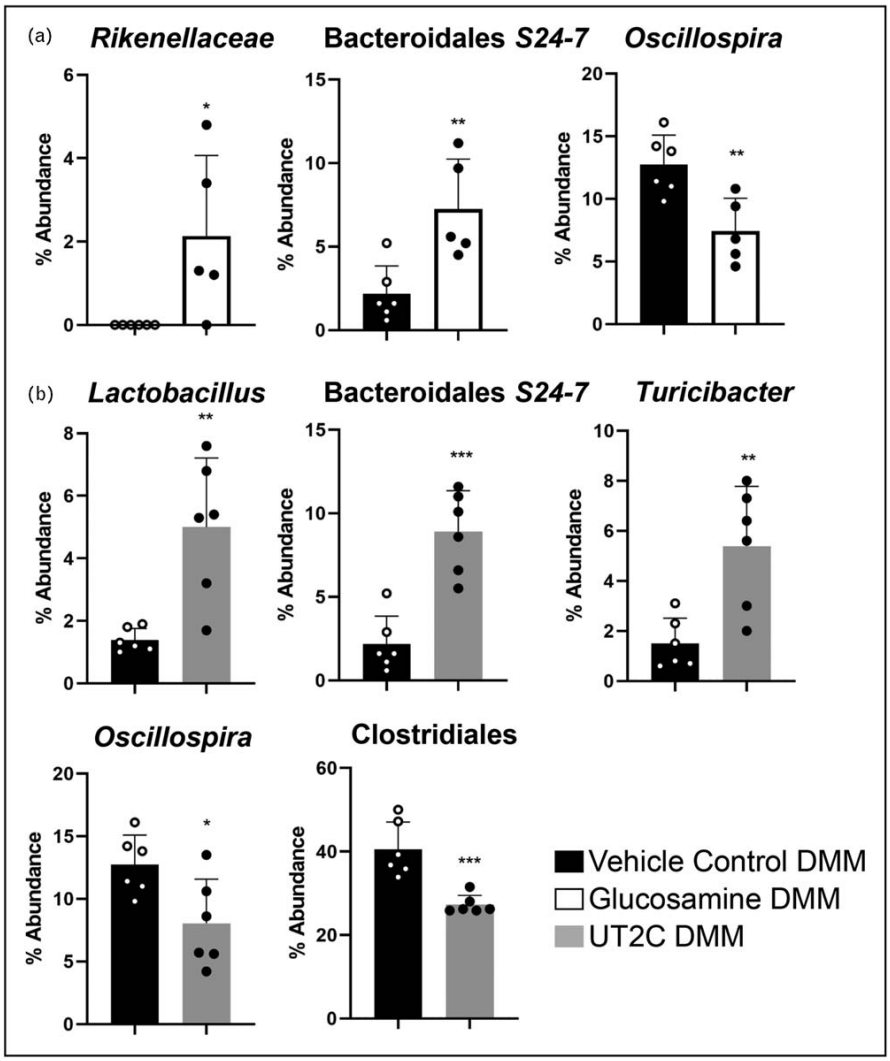

FIGURE 3. Individual operational taxonomic unit are significantly changed by supplementation with glucosamine or undenatured type II collage. (a) Supplementation with glucosamine for 14 weeks caused significant changes in abundance of three operational taxonomic unit compared with vehicle control. (b) Supplementation with undenatured type II collagen for 14 weeks caused significant changes in 5 operational taxonomic unit. Data shown represent mean % abundance ($n \geq 5$) ± SD. Significance was determined using Student's *t* test. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

in Firmicutes and decrease in Bacteroidetes has been associated with proinflammatory states in both humans and mice [62,63]. The change in the ratio may be linked with inflammation as an effect of both different responses to caloric intake and increased ability to extract calories from food [64,65]. Related to this, we found an increased Bacteroidetes/Firmicutes ratio in the microbiome of mice fed either glucosamine or UT2C compared with the vehicle control (Fig. 2a), consistent with a potential anti-inflammatory shift.

Numerous differences at the level of individual operational taxonomic units (OTUs) were found when either glucosamine or UT2C supplemented mice were compared with the vehicle control (Fig. 2b). The family *Rikenellaceae* and the Bacteroidales family *S24–7* were significantly increased in mice fed glucosamine, while the supplement caused the opposite trend in an *Oscillospira* species (Fig. 3a). Dietary supplementation with UT2C produced even more changes in the abundance of individual OTUs than glucosamine when compared with the vehicle control. As with glucosamine, UT2C increased the abundance of *S24-7* and decreased the amount of *Oscillospira* (Fig. 3b). Both *Lactobacillus* and *Turicibacter* levels were increased in mice given UT2C

compared with vehicle control, and certain Clostridiales taxa decreased as well (Fig. 3b). Significantly, *Rikenellaceae* family members are reduced in proinflammatory models of induced colitis [66]. Their increased abundance in mice fed glucosamine may suggest that this family can contribute to a lower inflammatory state. The absence of a change in *Rikenellaceae* in the UT2C-fed mice may indicate a glucosamine-specific effect. We also found *Turicibacter* to be significantly increased in mice fed UT2C compared with vehicle control or glucosamine. This genus has been found to be expanded in RA [67] although it has also been shown to be reduced in HFDs [68].

The family *S24-7*, also known as Muribaculaceae and *Candidatus* Homeothermaceae, is a dominant member of the murine gut microbiome [61,69]. Like *Rikenellaceae*, they have also been shown to decrease in colitis [66], and here we demonstrate their increase with both glucosamine and UT2C supplementation. Recent work has shown that HFD, a cause of systemic inflammation, is associated with a decrease in both *Rikenellaceae* and *S24-7* [70]. In addition, *S24-7* are most often found in herbivores or omnivores with a high percentage of plant material in the diet; they ferment glucans to yield short-chain fatty acids that can have anti-inflammatory effects [61,71]. The biological impact of changes in *S24-7* may be more complex than a simple anti-inflammation as they are increased in diabetes-sensitive mice fed a HFD as well as after remission of induced colitis [61].

Nutraceuticals are long standing supplements implicated in joint health whose efficacy has been difficult to define with biological measures. Defining the mechanism of action has been problematic due to their relative inability to reach the joint. New findings presented here reveal that mice fed either glucosamine or UT2C supplements demonstrate changes in both joint health and microbiome. Tibia cartilage, uncalcified cartilage, and Safranin O^+ chondrocytes are all increased in supplemented mice compared with vehicle controls. Global changes in phyla, Bacteroidetes/Firmicutes ratio, and individual OTUs in mice given supplements mirror improvements in the joint. Together, these parallel phenotypes suggest that nutraceuticals may exert beneficial action on joint health through modulation of the gut microbiome; further proof of causal links will require deeper investigation.

CONCLUSION

Emerging research provides compelling evidence of a link between the gut microbiome and development of osteoarthritis. Most of the studies to date have identified interesting associations, with candidate interventions involving indigestible prebiotics providing the best evidence of a causal linkage between the gut and joints. Moving forward, the field must perform deep analysis of causation using fecal microbiota transplant methods and metabolomic study of molecular mediators produced by microbiota that can impact host biology and induce – or protect from – joint degenerative disease. In addition to defining the pathogenic role the gut microbiome plays in osteoarthritis, establishing this connection provides the opportunity for development of new and effective disease modifying osteoarthritis therapeutics. In fact, it is possible that the only commonly used intervention with evidence of improvement in osteoarthritis symptoms, namely nutraceuticals, act indirectly via effects on the gut niche.

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None.

Conflicts of interest

There are no conflicts of interest.

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

- ■ of special interest
- ■■ of outstanding interest

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The study is the first to suggest that gut dysbiosis manipulation through prebiotics can improve osteoarthritis outcomes. It used both 16S sequencing and RNA-Seq as outcome measures.

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