REVIEW

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The Microbiome and Musculoskeletal Conditions of Aging: A Review of Evidence for Impact and Potential Therapeutics

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ABSTRACT

Recently, we have begun to realize that the billions of microorganisms living in symbiosis with us have an influence on disease. Evidence is mounting that the alimentary tract microbiome, in particular, influences both host metabolic potential and its innate and adaptive immune system. Inflammatory states characterize many bone and joint diseases of aging. This prompts the hypothesis that the gut microbiome could alter the inflammatory state of the individual and directly influence the development of these common and burdensome clinical problems. Because the microbiome is easily modifiable, this could have major therapeutic impact. This perspective discusses evidence to date on the role of the microbiome and the highly prevalent age-related disorders of osteoporosis, osteoarthritis, gout, rheumatoid arthritis, sarcopenia, and frailty. It also reviews data on the effects of probiotics and prebiotic interventions in animal and human models. Despite suggestive findings, research to date is not conclusive, and we identify priorities for research to substantiate and translate findings. © 2015 American Society for Bone and Mineral Research.

KEY WORDS: AGING; NUTRITION; OSTEOARTHRITIS; OSTEOPOROSIS; SARCOPENIA

Introduction

The term gut microbiome (GM) describes the genetic material of the myriad microorganisms (often referred to as a community) within an animal intestine. Recent advances in genome sequencing technology reveal its remarkable complexity and point to its involvement in numerous traits and diseases. Collectively, gut microorganisms encode 150-fold more unique genes than the human genome.[1] Hence, the GM may be conceptualized as an additional organ undertaking a vast amount of metabolic reactions,[2] which influence normal physiology and host metabolism.[3] Humans and their microbiome have co-evolved over millennia and live intimately to mutual benefit.[4,5] The human GM starts developing at birth, is modulated by infant and adult diet, and may be disrupted by antibiotic administration (reviewed in Cox and Blaser[5]). The host inflammatory response is both educated and driven by interaction with gut microorganisms[6] and neonatal animals reared in sterility do not develop normal immunity or normal size.[7,8]

A wide range of diverse diseases and conditions lying outside the gut have been demonstrated to be associated with an abnormal or dysfunctional microbiome. Such conditions include type 2 diabetes mellitus (T2D),[9] obesity,[10,11] and cardiovascular disease.[12] This article reviews the current evidence on microbiota associations with musculoskeletal diseases of aging. Many of the clinical problems reviewed are associated with inflammatory change—either specific to disease or associated with age. Alterations in the microbiota provide plausible candidate mechanisms for driving both inflammation and altering the immune response and host metabolism, which in turn may modulate the development of musculoskeletal problems and frailty (defined below). Studies in these areas are challenging and have to be carefully planned to minimize confounding by factors such as host genetics,[13] age,[14] diet,[15] and the condition itself, as these factors are also important in shaping the gut microbiome. In addition, microbes may behave differently in different environments and geography. Thus, answering the tricky question of cause and effect in disease–microbiome associations remains to be answered unequivocally for many conditions, although transplanting beneficial human microbes into germ-free animal models has had successes in some traits like obesity.[13]

Therapeutics

The gut microbiome provides an attractive target for therapeutic intervention because it may be manipulated relatively easily. For example, it has been well established that antibiotics alter the delicate balance of microorganisms, and changes can be stable and long lasting.[16,17] Clinical applications of this method include use of the nonsystemic broad-spectrum antibiotic

Received in original form October 26, 2015; revised form December 1, 2015; accepted December 11, 2015. Accepted manuscript online December 16, 2015.
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Journal of Bone and Mineral Research, Vol. 31, No. 2, February 2016, pp 261–269
DOI: 10.1002/jbmr.2765

rifaxamin in hepatic encephalopathy, diverticulosis, and irritable bowel disease.[18,19] Dietary manipulations, such as energy restriction,[20] high meat/fat diet,[21] and changes in fiber also modulate the microbiome. Effects are generally transient, but some longer-term influence may be possible if diet is sustained.[22]

Another method of altering the microbiome is through the use of pro- and prebiotics. A probiotic is a living organism found in food and dietary supplements that may improve the health of the host beyond its inherent basic nutritional content.[23] Prebiotics are ingredients in fruits and vegetables, such as complex carbohydrate fibers, that alter the composition or activity of the microbiome in a way that may confer health benefits to the host.[24]

Two substances currently meeting the criteria for classification as a prebiotic (Box 1) are inulin and trans-*Galacto-oligosaccharides*,[24] and more are anticipated.[25] Such prebiotics can be found in naturally occurring fruits and vegetables and produced artificially. Inulin, for example, is found in onions, leeks, asparagus, Jerusalem artichokes, chicory root, and bananas. The underlying mechanisms behind any health-promoting effects of probiotics and prebiotics are not yet proven but are likely to include alterations in the gut flora influencing metabolites produced, releasing short-chain fatty acids, and modulating the immune system; increased solubility and absorption of minerals; and enhanced barrier function.[26]

Box 1 : Criteria for classification as a prebiotic

a. Show resistance to gastric acidity, hydrolysis by mammalian enzymes, and GI absorption
b. Be fermented by intestinal microbiota
c. Selectively stimulate the growth and activity of intestinal bacteria, in vitro and in vivo experiments

A more radical treatment option is fecal transplantation or bacteriotherapy—now routinely used in 500 US centers after three randomized controlled trials (RCTs) for persistent *Clostridium Difficile* infection.[27] Donor or engineered microbial transplantation could also treat other microbe-associated diseases, although firm RCT evidence of efficacy is currently lacking. Greater understanding of the mechanism of action, efficacy, and safety are undoubtedly needed,[28] but intervention studies targeting the gut microbiome in age-related bone and joint disease will provide fascinating insight and will determine whether there is a clinical window for microbiome manipulations to reduce severity of diseases in the elderly.

Frailty

Despite a recent exponential increase in human life span, the number of years with good health-related quality of life (health span) has not kept pace.[29] The resulting increase in morbidity is not through single organ disease but largely explained by loss of physiological reserve capacity in multiple systems simultaneously, resulting in reduced resistance to stressors with increasing age. This shrinking of the homeodynamic space has been characterized as frailty,[30,31] a multidimensional state, which, importantly, is predictive of adverse health events, such as disability, hospitalizations, dependency, institutionalization, and mortality (Box 2). There is a strong relationship between frailty and bone and joint diseases: Not only are older individuals with bone and joint disease frail, but also frailer individuals have different response to illness and treatment. By 2050, it is estimated that 1.2 billion people worldwide will be frail.[32,33]

Box 2 Summary: Frailty and sarcopenia

Frailty is the age-related loss of reserve capacity in multiple systems simultaneously, which results in reduced resistance to stressors at increasing age.

Many older individuals with bone and joint disease are also frail

Frailty has been associated with alterations in the microbiome, in particular core butyrate producing commensals.

Sarcopenia is a condition of muscle loss and decreased performance.

A mouse model of sarcopenia appears to be impacted by specific *Lactobacillus* strains

There is now good evidence that frailty is associated with low-grade chronic inflammation, especially in women,[34] contributed to by the age-related increase in systemic inflammation ("inflamm-aging"[35]). Seventy percent of the body's lymphocytes reside in the gut-associated lympoid tissue,[36] and, owing to its folded structure, the alimentary tract constitutes the largest interface with the external world at 30 to 40 m^2,[37] a magnitude larger than the skin surface ($\sim$2 m^2). Therefore, alterations in the GM could play a role in the development of frailty.

Surprisingly few published studies have investigated the association. The Eldermet study in Cork showed that the fecal microbial diversity of 178 older adults appears to vary with level of health dependency—with patients in long-stay continuing-care settings having less diverse flora than short stay or community-dwelling older adults. Dietary factors, which may vary by health care setting, appeared to drive differences in microbial patterns.[15] A very small study of frailty and the microbiome showed significant differences in the abundance of 17 key gut microbes between 10 highly frail and 13 "low frail" individuals sharing the same diet in a single-care home.[38] Data from TwinsUK show association between microbiota patterns and specific bacteria with prefrailty in a community-dwelling population, which persists even after adjustment for diet. In particular, there may be a negative relationship between frailty and *Faecalibacterium Prausnitzii*, a bacterium with known anti-inflammatory effects.[39] Cross-sectional data in Eldermet[40] and our own unpublished results show that subjects having a diverse Mediterranean-style diet possess a more diverse and "healthy"—meaning rich and varied—gut microbiota.

Sarcopenia

Sarcopenia is frequently associated with frailty and bone and joint diseases and is characterized by progressive and generalized loss of skeletal muscle mass and strength, leading to physical disability, increased risk of falls, impaired ability to perform activities of daily living, loss of independence, and increased risk of death (Box 3).[41] The presence of sarcopenia makes rehabilitation from bone and joint diseases and orthopedic surgery much more problematic.

Muscle wasting occurs in several other pathological conditions, such as cancer, chronic heart failure, chronic infection,

Box 3 The European Working Group on Sarcopenia in Older People (EWGSOP) Criteria for diagnosis of Sarcopenia

1. Low muscle mass
   AND
2. Low muscle strength
   OR
3. Low physical performance

and malnutrition. Inflammation and inappropriate nutritional state are postulated to be important common mechanisms.[42] In a prospective study of nearly 1000 older individuals (mean age 74.6 years) higher levels of serum interleukin (IL)-6 and C-reactive protein (CRP) increased the risk of muscle strength loss over a 3-year period.[43]

One recent animal study suggests a relationship between muscle wasting and alterations in the gut microbiome. Muscle wasting induced by a model of acute leukemia in mice was reduced by orally supplementing the mice with specific *Lactobacillus* species.[44] The Authors suggest that gut microbiota may influence muscle physiology through altering amino acid bioavailability; influencing metabolites such as bile acids; and modulating production of pro-inflammatory cytokines.[42]

The microbiome may also be important in sarcopenic obesity. Although this condition lacks universal diagnostic criteria, it consists of increased adiposity, redistribution of fat, low-grade chronic inflammation, and fat infiltration into muscle (which decreases muscle strength and function).[45] A great deal of research has explored the role of the gut microbiome in obesity. Human studies show that overweight individuals of all ages have a less diverse microbiota and have significantly higher levels of gut bacteria that promote inflammation and weight gain compared with normal-weight individuals.[45]

Osteoporosis

Osteoporosis is characterized by a decrease in bone strength, itself a combination of bone density and bone quality, which harbingers an increased risk of bone fracture[46] (Box 4). Elevated inflammatory markers, such as high-sensitivity C-reactive protein (CRP), have been consistently associated with low bone mineral density, elevated bone resorption, bone loss, and increased fracture risk.[47–50] Furthermore, numerous inflammatory diseases are associated with osteoporosis,[51] including rheumatoid arthritis (RA) and inflammatory bowel disease (IBD). In both conditions, bone loss is regulated by pro-inflammatory cytokines.[51,52] Immune system alterations may also contribute to the loss of bone mass seen in postmenopausal women, where withdrawal of estrogen results in increased formation and prolonged survival of bone-resorbing osteoclasts (OCs).[53]

Box 4 Summary: Osteoporosis

Osteoporotic fractures are a major source of morbidity and mortality.

Osteoporosis has a substantial inflammatory component that may be affected by changes in the microbiome.

Probiotics and prebiotics have been linked to improvements in bone density in human and animal studies, indicating that the microbiome may be an important therapeutic target in osteoporosis.

Increasingly, immune-inflammatory axes are thought to be influenced by the host microbiome.[54] Compared with controls, germ-free (GF) mice have been shown to exhibit *increased* bone mass alongside fewer OCs in trabecular bone and a reduced number of $CD4^+$ T cells and OC precursor cells in bone marrow.[55] Colonization of GF mice at 3 weeks of age with normal mouse gut microbiota normalized the findings, leading the authors to suggest that altered immune status of the bone leads to increased OC bone resorption.[55]

In mice, subtherapeutic levels of antibiotics have been shown to alter the microbiome and increase bone mineral density (as well as body mass) in both early life and adulthood.[56,57] Specific probiotics and prebiotics have been shown to increase bone mass in both male and ovariectomized female (Ovx) rodent models (mimicking postmenopausal estrogen deficiency).[58–63] In both cases, bone improvement has been linked to changes in the microbiome.[64–66] This work indicates that such supplements may alter the immune status in bone, resulting in attenuated bone resorption. Ovx mice treated with *Lactobacillus reuteri* (a probiotic) showed significantly decreased bone RNA levels of Trap5 and receptor activator of NF-κB ligand (RANKL) (osteoclast activation and bone resorption markers) as well as decreased osteoclastogenesis.[66] In another study, mice fed with *Lactobacillus* strains then ovarectomized were protected against ovarectomy-stimulated bone loss and showed greater bone mineral content and reduced expression of inflammatory cytokines, TNFα and IL-1β, with increased expression of osteoprotegerin (OPG), a potent inhibitor of osteogenesis, compared with controls.[67]

Prebiotics influence mineral bioavailability from the diet. Studies in humans show that prebiotics increase calcium absorption in both adolescents and postmenopausal women,[68,69] and one demonstrated accompanying increased bone mineralization [70] Adolescents randomly assigned to receive mixed short- and long-chain inulin-type fructans had significantly increased whole-body mineral content and whole-body bone mineral density, compared with those given placebo. The authors proposed this was most likely because of changes in calcium absorption; however, changes in GM composition and the immune response may have been responsible.[53,70]

The effect of galacto-oligosaccharides (GOS) on calcium absorption and, importantly, the fecal microbiota has been examined in adolescent girls.[71] Levels of beneficial fecal bifidobacteria were significantly increased in a dose-dependent manner. Calcium absorption also increased, but this was independent of dose. The same group showed microbiota changes accompanying increased calcium absorption in a low-calcium diet in adolescent children of both sexes taking a soluble maize fiber compared with control but no changes in markers of bone turnover.[72]

Diets rich in soya have been associated with a reduced risk of osteoporosis, especially in Japanese populations. This effect has been attributed to (S)-equol produced from the soya isoflavone daidzein by bacterial rather than human enzymes.[73] Geographical and ethnic variation in the response to soy diets (such as for breast cancer) may be explained by differing microbiota in Western and Japanese populations.[74] This underlines the cautionary principle that dietary interventions may have different effects in different populations owing to differences in microbial communities.

Osteoarthritis

OA is classically thought to be a noninflammatory arthropathy involving cartilage and bone remodeling (Box 5). However, some studies have consistently demonstrated a degree of inflammation at all stages of the disease process.[75,76] Indeed, cytokines, chemokines, and other inflammatory mediators are produced locally by the synovium and chondrocytes and are detectable in OA synovial fluid.[75,76] Furthermore, PCR analyses of OA synovial fluid and synovial tissue have detected bacterial DNA, raising the possibility that live bacteria or bacterial products are present in the joint during disease progression.[77,78]

Box 5 Summary: Osteoarthritis

Osteoarthritis is the most common disorder of the musculoskeletal system.

It is a debilitating disease with no cure and limited treatment options.

New treatments with fewer side effects are desperately needed.

The literature considering the microbiome and the use of pro/prebiotics in OA is sparse but intriguing, and more studies are required.

A study in an animal model of OA should stimulate further research: oral administration of *Lactobacillus casei* (a probiotic) alone or alongside type II collagen (CII) and glucosamine (GS) (a candidate prebiotic) was given to arthritic rats (knee intra-articular monosodium iodoacetate [MIA] model).[79] *L. casei* appeared to have synergistic action with CII and GS, effectively reducing pain, cartilage destruction, and lymphocyte infiltration more than the treatment with GS and CII together or separately. Co-administration led to reduced expression of numerous pro-inflammatory cytokines and matrix metalloproteinases and upregulation of anti-inflammatory cytokines IL-10 and IL-4. This study is intriguing, but the protocol for rat pain assessment does not seem to have been blinded, and there was no analyses of the gut microbiome. Similar studies of probiotics on collagen-induced arthritis (CIA) in rats, a standard model of RA (see Box 6), have produced results comparable to those of nonsteroidal anti-inflammatory drugs,[80] suggesting that, irrespective of the underlying mechanism of joint inflammation, probiotics may be beneficial. Human studies are awaited with interest.

Box 6 Summary: Rheumatoid Arthritis

RA is an autoimmune inflammatory arthritis

Changes in the oral microbiome, in particular *Porphyromonas spp*, have been implicated in worsening of symptoms in mouse models and humans

Alterations in the gut microbiome, in particular in *Prevotella spp*, associate with RA, but disease stage and genotype appear to moderate associations seen.

The effect of green-lipped mussel (GLM) extract and glucosamine (GS), two candidate prebiotics, on the microbiome and OA has been explored in a nonblinded randomized clinical trial of 38 human subjects with knee OA.[81] Each participant received either 3000 mg/d of whole GLM extract or GS orally for 12 weeks, and stools were cultured at baseline and week 12 of follow-up. Changes to the microbiome were noted in both groups—but did not reach significance—but because a substantial proportion of gut bacteria are not culturable at present, studies with sequence-based microbiota assays are urgently needed. There is much debate about the efficacy of GLM and GS,[82–85] which may in part be owing to difference in baseline microbiota between studies and individuals, because both substances are metabolized in the colon by gut bacteria.[81]

One prevalent and costly outcome in both osteoarthritis and osteoporosis is surgical intervention and joint replacement, and skin microbiota may play an important role in wound healing. The use of oral vancomycin (which has poor systemic absorption) delayed wound healing and altered skin expression of promoters of keratinocyte proliferation IL17 and RegIIIγ in mice,[86] possibly through alteration of the gut microbiome. Most studies in human wound healing have looked at diabetic or chronic wound microbiota.[87] Studies of the skin and alimentary microbiota predictive of wound healing in the surgical population are warranted, especially because consumption of antibiotics is high in older, frailer individuals.

Rheumatoid Arthritis (RA)

Rheumatoid arthritis (RA) is an autoimmune disease affecting the synovium and cartilage, often with bony erosions. The epidemiology of RA is shifting and age of onset of rheumatoid arthritis is increasing,[88] only partly because of population aging (Box 6). Older patients often manifest more severe disease, despite shorter duration of symptoms at presentation,[89] but the reasons for this are not clear. Components of intestinal microbiota direct key aspects of host immunity, in particular effector T-cell differentiation,[90] which may impact susceptibility to autoimmune disease in general and RA in particular.[91] The intestinal microbiota are also known to change with age.[14]

RA has long been associated with periodontal disease and recent work on the oral microbiome focus on oral *Porphyromonas* spp (reviewed in Brusca et al.[92]). This species is unique in its ability to citrullinate proteins, which may then be involved in augmenting autoimmunity; notably, antibodies against citrullinated proteins (ACPAs) are one of the hallmarks of seropositive RA and have been shown to be pathogenic. Animal studies of RA (CIA model) support that *Porphyromonas* have an accelerating effect.[93,94]

Genetic risk may be moderated by alterations in the microbiome. *Prevotella copri* in stool has been associated with the new onset nontreated RA in humans (but not treated RA).[95] Here HLA DRB1 carriers had lower levels of *P. Copri*, which the authors suggest might indicate a lower threshold for disease presentation. More work needs to be done to investigate whether, in humans, microbiota differ by HLA type as has been found in murine species.[96]

Gout

Both acute gouty arthritis and tophaceous gout are characterized by the presence of monosodium urate in both affected and unaffected joints (Box 7). The inflammation and morbidity associated with gout is a consequence of the activation of a cytokine cascade, which it now appears has IL-1β as central controller.[97] Recent evidence from animal studies suggests the gut microbiome modulates the response to gout crystals via inflammasome assembly and IL-1β production. Germ-free mice manifest a blunted inflammatory response to injection of

Box 7 Summary: Gout

Gout is an acute and chronic highly inflammatory crystal arthritis

Much of the pathology in gout is mediated by the exuberant inflammatory response

Animal studies show moderation of inflammation by the gut microbiome

monosodium urate compared with wild-type mice. This effect was normalized by recolonization of their gut flora or by acetate supplementation (the short-chain fatty acid produced by gut commensals with greatest systemic distribution).[98] In the same experiment, wild-type mice treated with antibiotics and mice deficient in a key short-chain amino acid receptor (GPR43) also showed reduced immune response. GPR43 expression is increased in immunocytes in the ileum and colon[99] and appears to mediate the effect of short-chain fatty acid production by commensal bacteria on the inflammasome. The same pathway may be involved in other inflammatory responses—for example, response neutrophil chemotaxis and response to bacterial infection.[100] Contrary to gout models, in K/BxN serum-induced autoimmune arthritis, GPR43-deficient mice produced more severe and nonresolving inflammatory response than that found in the wild type.[101]

Human epidemiological studies have long implicated dietary changes in the onset of acute gout. In particular, animal-derived purines, alcohol, and fructose appear to exacerbate gout, and low-fat dairy products, coffee, and vitamin C appear to have a protective effect.[102] Assuming a causal association, the mechanism by which these take place is not clear. Studies are needed to examine whether the microbial metabolism of these dietary factors associates with urate levels and acute gout in humans and whether the microbiome mediates the response to diet in humans or animal models of gout. Again, this offers a very attractive route to therapy, potentially low in systemic side effects and easy to administer.

Drugs and the Microbiome

Gut microbes have a major role in metabolism of several drugs commonly used in the management of musculoskeletal diseases of aging and are a source of much commercial interest for new therapeutics (Box 8). First, common analgesic medications, still a mainstay of symptom control, are influenced by an individual's gut microbiome. Gut microbes producing p-cresol, which competes for human sulphonating enzymes, directly affect paracetamol metabolism potentiating toxicity.[103] Moreover, as p-cresol is itself a metabolite of tyrosine, manipulation of dietary tyrosine also may potentiate paracetamol toxicity. Second, the gastric and intestinal toxicity of nonsteroidal anti-inflammatory drugs (NSAIDs) is influenced by a bacterially derived enzyme β-glucuronidase. In mice, inhibitors of this bacterial enzyme markedly reduced ulceration associated with a variety of NSAIDs.[104] Human studies showing manipulation in this pathway are urgently needed because they could have significant implications, especially in older adults where the risk of gastrointestinal bleeding is higher.

Box 8 Summary: Drugs in bone and joint disease and the microbiome

Gut microbes significantly affect the metabolism of drugs used in bone and joint disease, especially analgesics and plant-based flavonoids

Drugs used in bone and joint disease also have effects on the host microbiome

Epimedium-derived flavonoids have long been used in Chinese medicine for bone health, and a randomized double-blind placebo controlled trial in osteopenic postmenopausal women showed benefits in bone density over 12 and 24 months.[105] The investigators of this trial made careful effort to standardize the diets of the subjects, which may have reduced variance in the subjects' microbiota. Epimedii is significantly metabolized by gut microbes in rats, and therefore efficacy may be altered depending on the host microbiota.[106] A more recent multicenter Chinese trial showed significant effect over 6 months only, but did not standardize diet during the duration of the study.[107] Studies are needed to determine whether this drug has potential in the context of a Western host microbiota and diet.

Other drugs used in bone and joint disease may be metabolized by the gut microbiota in in vitro models, but this will only have effect on treatment when absorption is delayed until the colon. For example, gut microbes readily metabolize corticosteroids, but predisolone (absorbed proximally) is unaffected, whereas oral budesonide passes through and is readily metabolized in the colon. Therefore, gut microbial metabolism may have a role in response to budesonide treatment in inflammatory bowel disease.[108] Absorption of some drugs, such as the osteoporosis treatment strontium, are significantly affected by diet—especially alginates,[109] which are in turn metabolized by specialized microbes—leading to a complex interaction between the drug, host-specific diet, and microbiota profile. Initial epidemiological evidence found associations between changes in the oral microbiome and the severe bisphosphonate side effect osteonecrosis of the jaw, but recent evidence suggests that this relationship is not directly causative but rather reflects alterations in systemic immunity.[110]

Most of the examples of drug microbe interactions discussed above involve the alteration of drug metabolism by the microbes. However, drugs may influence bone and joint disease or exhibit side effects, through altering the GM itself. For example, vitamin D interacts with the gut microbiome—especially in the proximal gut, increasing microbial diversity and modulating T-cell numbers,[111] and this may be part of its mechanism of action. On the other hand, early animal work suggests that methotrexate influences microbial abundance and diversity, and it appears likely that this mechanism contributes to the drug-associated mucositis found at high doses.[112] In addition, the largest study to date in >1500 TwinsUK individuals has confirmed earlier suspicions that proton pump inhibitors (PPIs) significantly alter the GM, with oral flora being carried through the gastrointestinal tract.[113] Mounting evidence indicates that PPIs are associated with an increased risk of fracture.[114] The mechanism is unclear,[115] but interaction with the gut microbiome is a novel possibility.

Conclusion

There is much scope for further investigation of the impact of the human microbiome on bone and joint disease found at

older age, particularly the gut microbiome. The microbiome is a highly plausible target for modulation of diseases of aging owing to its close relationship with the innate and adaptive immune systems. It should not be considered in isolation because of the recognized influence of host genetics,[13] geography, diet, and other factors.

To date, some of the best data relating the microbiome to bone disease is in osteoporosis and RA. Other diseases, in particular osteoarthritis, have received little attention to date, despite some promising suggestive findings. Clearly, there is room for well-planned studies in these areas. However, even in the best cases, studies are limited by three main issues. First, the methodology of microbiota research is still a work in progress, and the optimal methods for gaining clinically relevant samples and how they should be sequenced and assigned are yet to be established. Compositional (taxonomic) approaches used in most of the studies reviewed here do not take full account of the functional capacity of microbiota, which may have more biological significance. Few studies to date make use of comprehensive but expensive metagenomic (shotgun) data, which identifies around 80% of all species of microorganism (including fungi, archea). Most published studies use primers for one highly variable gene, the 16SrRNA, which is used for bacterial identification. Taxonomic assignation is only as good as the library used and predicted functions rely on accurate and relevant reference sequences. Metatranscriptomic analyses of intestinal mucosal samples are needed to inform on which genes are actually being expressed and microbiome metabolomics is also in its infancy.

The second issue is that most studies to date have focused on the microbiome of the distal large intestine as revealed by stool samples. This focus stems from the fact that up to 70% of human inflammatory cells are present in the gut-associated lymphoid tissue,[36] and feces are relatively easy to collect. However, there may be significant differences between microbiota along the course of the intestine[116] from the mouth to the colon and at the mucosal interface compared with the lumen.[117]

The third issue is to what extent studies in model animals translate to humans. So far, most of the evidence for a benefit of probiotics is in rodents. In humans, probiotic studies have only shown evidence in randomized controlled trials in very old or young populations or the severely ill. Moreover, the rodent natural microbiota are very different from that of a human, and many studies attempting to "humanize" the murine microbiome have only limited success for short periods, underlining the power of the host to determine its own microbiota. Human variability and underlying genetic variance will make comprehensive clinical studies essential to perform in humans.

Despite these issues, microbiome markers are likely to form part of future multi-omics panels to predict disease risk and indicate prognosis (Box 9). Identifying high-risk patients with low-diversity microbiome could plausibly lead to simple and safe dietary or therapeutic interventions.

Although more trials in humans are needed, because of the tight relationship between host and microbe and wide variability, these will be problematic and underpowered until we understand the impact of specific host genotypes on the microbiota, and integrate "omic" wide analyses of the impact on metabolomics and immunophenotyping. Despite the lack of large-scale human data and our current limited understanding of the gut microbiome, the example of how fecal transplants have rapidly become mainstream life-saving treatments in *C. Diff* infections shows the potential for pragmatic use of gut microbes as agents for good. This is a fast-moving field and has great potential in improving the health of elderly populations with bone and joint conditions.

Box 9 : Critical questions for the future

- Do specific microbiome alterations moderating bone and joint disease and sequelae in animal studies translate to humans?
- How can they be modified in a sustained manner required in chronic diseases?
- How do individual differences in the microbiota alter the therapeutic responses to common drugs and nutritional interventions used in bone and joint disease?
- Should we be prescribing prebiotic foods and probiotics in frail patients?
- Should we be routinely testing our patients for disordered gut microbiota?

Disclosures

All authors state that they have no conflicts of interest.

Acknowledgments

CJS is funded by a fellowship grant from the Chronic Disease Research Foundation. The TwinsUK study was funded by the Wellcome Trust, European Community's Seventh Framework Programme (FP7/2007-2013). The study also receives support from the National Institute for Health Research (NIHR)-funded BioResource, Clinical Research Facility, and Biomedical Research Centre based at Guy's and St Thomas' NHS Foundation Trust in partnership with King's College London. SNP genotyping was performed by the Wellcome Trust Sanger Institute and National Eye Institute via NIH/CIDR.

Authors' roles: CJS and SB performed literature reviews and drafted the manuscript. FKMW and TDS reviewed the manuscript and approved the final draft.

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