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The Microbiome: A Heritable Contributor to Bone Morphology?

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Abstract

Bone provides structure to the vertebrate body that allows for movement and mechanical stimuli that enable and the proper development of neighboring organs. Bone morphology and density is also highly heritable. In humans, heritability of bone mineral density has been estimated to be 50-80%. However, genome wide association studies have so far explained only 25% of the variation in bone mineral density, suggesting that a substantial portion of the heritability of bone mineral density may be due to environmental factors. Here we explore the idea that the gut microbiome is a heritable environmental factor that contributes to bone morphology and density. The vertebrae skeleton has evolved over the past ~500 million years in the presence of commensal microbial communities. The composition of the commensal microbial communities has co-evolved with the hosts resulting in species-specific microbial populations associated with vertebrate phylogeny. Furthermore, a substantial portion of the gut microbiome is acquired through familial transfer. Recent studies suggest that the gut microbiome also influences postnatal development. Here we review studies from the past decade in mice that have shown that the presence of the gut microbiome can influence postnatal bone growth regulating bone morphology and density. These studies indicate that the presence of the gut microbiome may increase longitudinal bone growth and appositional bone growth, resulting differences cortical bone morphology in long bones. More surprising, however are recent studies showing that transfer of the gut microbiota among inbred mouse strains with distinct bone phenoytpes can alter postnatal development and adult bone morphology. Together these studies support the concept that the gut microbiome is a contributor to skeletal phenotype.

Keywords

Microbiome; Bone; Development; Growth; Evolution

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1.0 Introduction

Bone is among the most visually compelling components of vertebrate anatomy and provides the most compelling information in the fossil record. Functionally, bones provide structure for the body as well as contact points for tendons and muscle that enable movement. Appropriate prenatal and postnatal development of bone allows for the generation of mechanical stimuli that help shape surrounding tissues and organs.

Bone morphology and density are determined by a combination of genetic and environmental factors. Multiple lines of evidence suggest that bone morphology is heritable. In humans, bone mineral density (BMD), as measured using dual energy x-ray absorptiometry (DXA), is the most widely used quantitative metric of bone mass. Bone mineral density increases during growth and reaches a peak value in the third decade of life. Bone mineral density is then maintained at this peak for several years prior to the initiation of age-related decline. The accumulation of bone mineral density during adolescence is the most influential determinant of adult bone mineral density [1]. In humans, the accumulation of bone mineral density tracks during growth; growing children with low bone mineral density tend to have low bone mineral density at later ages [2]. The heritability of adult bone mineral density has been reported to range from 50-80% [3], indicating that bone mineral density is highly heritable trait. However, the combined information of genome-wide association studies together explain only ~25% of the variance in bone mineral density among individuals [3]. The difference between overall heritability of bone mineral density and the degree to which genetics predicts bone mineral density suggests that environmental factors may play an important role in determining bone phenotypes.

The gut microbiome is an environmental factor that can influence organs throughout the body and has a highly heritable component [4]. The mammalian microbiome consists of the microbial communities that inhabit surfaces of the body [4,5]. The majority of the mammalian microbiome is present in the gastrointestinal system. The mammalian gut microbiome consists of hundreds of distinct microbial species (bacteria, archea, viruses, single celled eukaryotes) interacting with one another and with host cells at the gut endothelial barrier. The body is first colonized by microbes soon after birth. Over the first few years of life the composition of the gut microbial community fluctuates considerably until achieving a relatively stable composition [6–8]. The gut microbiome is also heritable: maternal transfer of the microbiome soon after birth is among the most influential contributors to the establishment of the gut microbiome [9]; and later in life components of the gut microbiota are transferable through close contact such as that occurring within households and due to familial dietary habits [8,10]. The composition of a mature gut microbiota can fluctuate on an hourly or daily basis due to variations in diet [11,12]. However, the overall composition of an established gut microbiota are robust to perturbations; the vast majority of the microbial composition returns to its prior state following a mild or temporary perturbation [5,11,13]. Hence, the composition of the gut microbiota is partially heritable and, once established, does not change substantially without a large or prolonged stimulus. That the gut microbiota is established at an early age suggests that heritable components of the gut microbiota may contribute to the patterns of bone mass accrual that determine adult bone morphology and density.

This review explores the idea that the heritable component of the gut microbiome has the potential to influence the adult bone phenotype. We first discuss the heritability of the gut microbiome across species, examining the interactions between the gut microbiome and evolution of host organisms, and then review recent studies in mice describing how differences in the composition of the gut microbiome during growth can lead to distinct bone phenotypes at skeletal maturity.

2.0 The Microbiome and the Host

Bacteria represent the earliest cellular life on Earth, predating vertebrates by more than 3 billion years. The evolutionary history of vertebrates, beginning ~500 million years ago, has been shaped continuously by selective forces imposed by bacteria. As vertebrate body sites diversified, novel ecological niches arose and were quickly filled by bacteria from the external environment [14,15]. These early bacterial inhabitants of vertebrates had profound effects on their hosts, driving immunological and morphological adaptations. In turn, selection within vertebrate hosts drove specialization of once free-living bacterial lineages, generating host-associated symbionts spanning the parasitism–mutualism continuum. Today, all vertebrates are colonized by complex assemblages of bacteria containing hundreds of species.

Historically, the effects of bacteria on vertebrate evolution have been studied primarily in the context of pathogenesis: gut bacterial pathogens can induce phenotypic plasticity in individual hosts [16], contribute substantially to mortality in host populations [17], and drive the evolution of complex defense mechanisms in host species[18,19]. However, current evidence suggests that most bacterial lineages harbored by vertebrates are benign, or even beneficial [15]. For example, most bacteria associated with vertebrates reside in the gastrointestinal tract, where densities can reach ~10^{11} cells per milliliter, yet relatively few of the constituents of this gut microbiota are known to cause disease in their hosts. Instead, experimental evidence indicates that the gut microbiota beneficially contributes to the development of a wide range of vertebrate phenotypes. Manipulation of the gut microbiota in germ-free and gnotobiotic vertebrates ranging from zebrafish to mice have shown that the presence of bacteria is essential for normal metabolism, intestinal and immune differentiation, and neuroendocrine function [20–23].

There is emerging evidence that the beneficial effects that gut microbiota provide to vertebrates have resulted from millions of years of co-evolution between bacteria and hosts. Vertebrate guts are generally devoid of bacteria at birth, and the gut microbiota must be assembled anew in each host generation. Accordingly, the composition of the gut microbiota can be influenced by environmental variation, including host diet [11], geography [24], and temperature [25]. Nevertheless, the composition of the gut microbiota appears to be largely determined by host evolutionary history. Across vertebrates, gut microbiota variation among individual hosts has been observed to be significantly lower within host species than between host species [26], and differences in taxonomic composition between the gut microbiota of vertebrate species tend to reflect the evolutionary relationships of the host species, an observation that has been termed ‘phylosymbiosis’ [27]. These associations between the composition of the gut microbiota and host phylogeny have been observed

across diverse vertebrate clades, including primates, rodents, carnivores, reptiles, and birds [24,26,28,29]. Moreover, in rodents, compositional differences (based on 16S rRNA gene amplicon sequencing) between the gut microbiota of host species have been found to remain stable in animals housed under shared laboratory conditions and diets [26,29]. These studies have also shown that the association between host phylogenetic history and the composition of the gut microbiota remains stable in a shared laboratory environment. Together, these observations suggest contributions of innate differences between host species to the composition of the gut microbiota.

In addition to the observation that the taxonomic makeup of the vertebrate gut microbiota tends to reflect host phylogenetic history, recent work has discovered several examples in which individual constituents of the gut microbiota have co-diversified with host species. Co-diversification—the process in which two or more interacting lineages speciate in parallel—is the hallmark of ancient symbiotic associations. For example, *Bacteroides* and *Bifidobacterium* are two of the most abundant genera of bacteria in human and African ape gut microbiota [29], are vertically transmitted from mother to child [30], and contain species that have co-diversified with their hominid hosts over the past ~15 million years [31]. Phylogenies of *Bacteroides* and *Bifidobacterium* strains based on *gyrB* sequences match topologically the phylogeny of hominids, consistent with the maintenance of specific lineages of these genera exclusively within diverging host species over tens of thousands of host generations. These results indicate that gut bacterial genomes can diversify concordantly with host nuclear and mitochondrial genomes. In addition to these examples from hominids, evidence of co-diversification between gut microbiota constituents and host species has also been observed across a diversity of other mammalian species and may extend into other vertebrate clades [32,33].

The long-term stability of the relationships between gut bacteria and vertebrates affords opportunities for co-evolution, in which populations of symbionts and hosts reciprocally adapt to one another. A history of co-evolution between hosts and gut microbiota is supported by recent experimental observations that vertebrate development in some cases depends on the presence of a host-species specific gut microbiota. For example, transplantation of rat (*Rattus rattus*) or human (*Homo sapiens*) gut microbiota into germ-free house mice (*Mus musculus domesticus*) stunts the differentiation of T-cells in the recipient mice relative to the transplantation of house-mouse gut microbiota [22] . Similarly, transplantation of gut microbiota from Gairdner's shrewmouse (*Mus pahari*) into germ-free house mice stunts host growth rate relative to transplantation of house-mouse gut microbiota [34]. Therefore, host-species specific gut microbiota of house mice affect house-mouse postnatal development, and differences among host-lineage specific gut microbiota are in some cases sufficient to generate phenotypic variation among vertebrates. Together, these observations raise the exciting possibility that variation in the gut microbiota may contribute to heritable variation in phenotypes among vertebrate lineages that cannot be readily explained by host genetic factors.

3.0 The Microbiome and the Adult Bone Phenotype

The ability of the gut microbiome to influence bone morphology has been recognized since the first animal studies of oral antibiotics in the 1920-30s which reported alterations in whole body growth as well as bone length and morphology following chronic oral antibiotic dosing [35–37]. At the time, the influence of the microbiome on bone was attributed to the effect of the gut microbiome on nutrient absorption at the gut lining. Studies in the past decade, however, suggest the effect of the microbiome on bone is much more complex (Table 1).

Among the first recent studies to report bone phenotypes following alterations to the gut microbiome examined the effects of chronic, subtherapeutic doses of oral antibiotics (as are commonly applied to farm animals) on growth and overall health [38,39]. Cho and colleagues found that chronic, subtherapeutic dosing of oral antibiotics, starting at three weeks of age, led to some differences in whole body BMD (noticeable at six weeks of age but not at ten weeks of age). Cox and colleagues found low dose penicillin starting as early as birth (dosed to the dam before birth) led to measurable differences from untreated animals in terms of BMD (in female but not male mice) and differences in bone area (in male but not female mice) at 20 weeks of age. These studies were not designed explicitly to examine bone and used mouse DXA, a method of assessing bone morphology that has relatively low precision. For this reason, it is possible that the reported differences in bone (or lack thereof) could be related to limited statistical power.

More recent studies have been designed specifically to understand bone. In a study using micro-computed tomography to identify differences in cancellous bone morphology between germ-free and conventionally raised mice, Sjogren and colleagues found that germ-free female C57Bl/6 mice had increased trabecular bone volume fraction and increased metaphyseal trabecular volumetric bone mineral density as compared to conventionally raised animals [40]. The study was limited, however, to young, rapidly growing animals (7 weeks of age, see discussion below). Yan and colleagues [41] examined bone in germ-free mice and germ-free mice colonized with microbiota from conventionally raised mice. Germ-free mice had greater trabecular bone volume fraction than mice that had been colonized for one month (both groups examined at three months of age). Similarly, Yan and colleagues found that decimation of the gut microbial population using a cocktail of oral antibiotics for one month led to increases in trabecular bone volume fraction as compared to mice with unaltered gut microbiota (again examined at three months of age). Novince and colleagues observed greater bone volume fraction in germ free mice compared to specific pathogen free animals (although used only n=4/group)[42]. Together these findings would suggest that the presence of a gut microbiota leads to reduced trabecular bone volume fraction. However, Yan and colleagues also examined mice eight months after introduction of a gut microbiota, and found no differences in trabecular bone volume fraction as compared to completely germ-free mice (both groups examined at 10 months of age). Similarly, three different studies from the Pacifici group did not observe significant differences in the trabecular bone volume fraction between germ-free mice and mice colonized for four months (examined at five months of age) [43]; or between germ-free, conventionally raised and conventionally raised mice in which the gut microbiota was decimated by oral antibiotics (examined at 3

months of age) [44,45]. Guss et al. [46] and Luna et al. [47], used oral antibiotics to disrupt (but not decimate) the constituents of the gut microbiome for three months of life and did not observe differences in trabecular bone volume fraction at four months of age.

A potential explanation for the discrepancy between the cancellous bone phenotypes in young (less than 3 month old mice) and older mice is that the presence of a gut microbiota does not determine the bone phenotype at skeletal maturity but rather influences rates of bone growth. Mice undergo rapid bone acquisition in the first 3-4 months of age after which longitudinal bone growth slows drastically (but still continues) [48]. Trabecular bone is established in the growth plate and is therefore sensitive to modifications in matrix synthesis and remodeling within the growth plate. Changes in rates of bone acquisition may therefore generate differences in trabecular bone volume fraction when examined during periods of rapid bone growth (2-3 months of age) but would not necessarily result in differences in morphological phenotype at skeletal maturity. Consistent with this idea, Yan and colleagues reported noticeable differences in the thickness of the growth plate in three month-old conventionalized mice as compared to germ-free mice. That the effect of the gut microbiome on trabecular bone phenotypes differs between young, growing mice and skeletally mature mice highlights the importance of characterizing bone morphological phenotypes in mice after growth slows (3-4 months of age as recommended [48]). We conclude from these findings however, that the absence or depletion of the gut microbiota, while potentially influencing the acquisition of trabecular bone at the growth plate, likely has little effect on the amounts of trabecular bone present at skeletal maturity.

The presence of the gut microbiota also influences cortical bone, although it remains unclear if the effect increases or decreased metrics of cortical bone geometry. Sjogren and colleagues found that young (3 month-old) germ-free mice showed increased femoral cortical area as compared to conventionally raised mice [40]. Similarly, Li and colleagues reported significant increases in cortical area and cortical thickness at the femoral diaphysis in five month old germ-free as compared to conventionally raised mice [43]. However, in another study examining three month old mice, Li and colleagues found subtle increases in cortical thickness but not cortical area in germ-free mice (suggesting a potential increase in measures of cortical geometry in the absence of a gut microbiota), but observed clear reductions in cortical area and cortical thickness following decimation of the gut microbiota for one month using oral antibiotic cocktails (suggesting that removal of the gut microbiota reduces metrics of cortical geometry) [44]. In contrast, Schwarzer and colleagues found that young (two month-old) germ-free mice were much smaller than conventionally raised mice in terms of whole body mass, whole body length, whole bone length and femoral cortical area [49]. Similarly, Yan and colleagues found that adult (10 month old) germ-free mice had smaller endosteal and periosteal diameter at the femur midshaft and shorter whole bone length in adulthood than mice that had been conventionalized at two months of age, in part leading to their conclusion that exposure to the gut microbiota leads to a net increase in bone acquisition during life [41]. Guss and colleagues and Luna and colleagues found that disruption (but not decimation) of the gut microbiota in mice using narrow spectrum antibiotics from 1-4 months of age was associated with small but significant reductions in femur length [46,47]. Furthermore, Luna and colleagues observed large reductions in metrics of cortical bone morphology (cortical area, moment of inertia) following disruption

of the composition of the gut microbiota from 1-4 months of age [47]. These studies support the idea that the presence of the gut microbiota influences cortical bone morphology, but are not clear whether the effect increases or decreases metrics of cortical bone geometry. Perhaps the greatest challenge in interpreting these findings is that only two of the studies ([46,47]) adjusted for differences in body weight when comparing cortical bone morphology. The morphology of the cortical bone diaphysis is typically correlated with whole animal body weight [48] and when there are differences in body weight among study groups (as in the Schwarzer paper discussed above) adjustment for body weight is necessary for detecting differences in morphological traits that are not simply derived from overall animal size [48] (trabecular bone volume fraction discussed above is not strongly correlated with body weight). That being said, the majority of the studies indicate that, in the absence of a gut microbiota diaphyseal cortical bone is less robust and the long bones are potentially shorter.

The most direct demonstration that the gut microbiota influences a phenotype is to show that the phenotype can be transmitted through fecal microbiota transfer. Moeller and colleagues found that transfer of the gut microbiota from one rodent species to another led to impaired whole body growth [34], but did not directly measure bone. Inbred mouse strains provide a useful tool for testing this hypothesis within a species. Inbred mouse strains display drastically different bone morphology [48] at skeletal maturity and also known to harbor distinct gut microbial communities [50]. Tyagi and colleagues [51] used two inbred mouse strain with distinct bone phenotypes: the high bone mass mouse (C3H/HeN) and a low bone mass mouse (C57Bl/6). The gut microbiota from conventionally raised inbred mice was transferred into germ-free mating pairs. The resulting pups were thereby colonized with donor microbiota and raised to skeletal maturity (4 months of age). Mice receiving gut microbiota from the same inbred mouse strain showed expected bone phenotypes (for example, higher trabecular bone volume fraction and measures of femoral cortical geometry in the C3H/HeN mice as compared to the C57Bl/6 mice). However, C3H/HeN mice colonized by microbiota from the lower bone mass C57Bl/6 mice showed reduced trabecular bone volume fraction as compared to C3H/HeN mice (i.e. the trabecular bone phenotype was more similar to the donor mouse) and small but significant reductions in cortical area and cortical thickness (i.e. the cortical bone phenotype was slightly more like that of the donor mouse). However, C57Bl/6 mice receiving gut microbiota from the high bone mass (C3H/HeN) mouse did not display noticeably different trabecular bone volume fraction but did display small but significant increases in cortical area and cortical thickness as compared to mice with C57Bl/6 microbiota. One potential explanation for this finding is that the C57Bl/6 mice in the study were colonized with the inflammatory gut microbe known as segmented filamentous bacteria (SFB). It is possible that transfer of the C57Bl/6 gut microbiota into C3H/HeN mice led to reductions in trabecular bone volume fraction due solely as a result of the inflammatory SFB microbe and not any other components of the C57Bl/6 microbial community. Such a possibility would be consistent with the observation that C57Bl/6 mice receiving microbiota from C3H/HeN donors did not experience increased trabecular bone volume fraction. A potential limitation of the study is that evaluation of cortical bone did not adjust for body mass (see above). Despite these limitations, the reductions in trabecular bone volume fraction in the C3H/HeN mice

receiving microbiota from a low bone mass mouse (C57Bl/6) indicate that the adult bone phenotype can be modulated by the gut microbiota, especially in the presence of highly inflammatory commensals.

4.0 Conclusions and Areas of Future Investigation

Together the findings reviewed here demonstrate that the gut microbiota is a factor that has co-evolved with vertebrates and can have a profound effect on postnatal development including effects on bone length, morphology and density. Furthermore, the effects of the gut microbiome on bone appear to depend on the state of postnatal development at the time of modification of the gut microbiota (growing v. skeletally mature). These findings raise the possibility that differences in composition and/or function of the gut microbiome contribute to heritability of bone morphological phenotypes that are currently attributed to host genotype.

The observation that transfer of the gut microbiome from one mouse strain to another may also partially transfer the bone phenotype suggest that some of the distinct bone phenotypes among inbred mouse strains currently used to study genetics [48] are determined, in part, by differences in the composition of the gut microbiota [50]. If confirmed in subsequent studies, it may be necessary to control the composition of the gut microbiota when using inbred mouse strains to study the genetic determinants of bone morphology. It remains to be seen if the composition of the gut microbiome is correlated with bone mineral density in humans.

As the study of the microbiome and bone is in its early stages, there are many details that remain unknown. The studies reviewed here focused on drastic changes in the composition of the gut microbiota (presence/absence) although a few of the reports examined more subtle changes in the composition of the gut microbiota (disruption of the gut microbiota as opposed to decimation of the gut microbiota). Additionally, most of the studies to date have focused only on the long bones (femur in mice) and the effects of the gut microbiome on other parts of the skeleton has not been as well studied. Although the studies to date indicate an effect of the gut microbiome on growth patterns, none have directly addressed the role of the gut microbiome on postnatal bone growth through longitudinal and/or cross-sectional studies exploring the effects of the gut microbiome bone at different points in time during growth and maturation. Studies focusing on the effects of the gut microbiome on endochondral ossification at the growth plate and intramembranous ossification at the periosteal and endosteal surfaces at different ages in life are needed to provide insights on molecular mechanisms of bone development that are regulated by the gut microbiome and to identify potential targets for therapeutic interventions. Key questions include the degree to which the composition of the gut microbiome determines bone phenotypes at skeletal maturity as well as the points during growth when the gut microbiome has its greatest influence. Lastly, and most importantly substantially more work must be done to determine the underlying mechanisms that regulate bone morphology during growth and development. Several mechanisms have been proposed including the effect of the gut microbiome on immune cells at the gut lining that subsequently migrate to the bone marrow [52,53], vitamins produced by the gut microbiota [54] and the effects of circulating microbial proteins or metabolites such as lipopolysaccharide and butyrate [55,56].

Another exciting avenue for future work will be interrogating the degree to which the gut microbiota generates variation in bone among host populations and species. Humans and other mammals display immense variation in bone density, which affects processes ranging from fossilization [57] to fitness in extant populations [58], yet the relative contributions of environment and genetics to this variation remains poorly understood. Experiments in gnotobiotic hosts in which microbiota from divergent mammalian lineages are reciprocally transplanted under a common environment have the potential to directly measure the contribution of the gut microbiota to divergence in bone phenotypes.

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References

[1]. Gordon CM, Zemel BS, Wren TAL, Leonard MB, Bachrach LK, Rauch F, Gilsanz V, Rosen CJ, Winer KK, The Determinants of Peak Bone Mass, J Pediatr. 180 (2017) 261–269. [PubMed: 27816219]

[2]. Wren TA, Kalkwarf HJ, Zemel BS, Lappe JM, Oberfield S, Shepherd JA, Winer KK, Gilsanz V, Bone G Mineral Density in Childhood Study, Longitudinal tracking of dual-energy X-ray absorptiometry bone measures over 6 years in children and adolescents: persistence of low bone mass to maturity, J Pediatr. 164 (2014) 1280–5 e2. 10.1016/j.jpeds.2013.12.040. [PubMed: 24485819]

[3]. Rivadeneira F, Uitterlinden AG, Genetics of osteoporosis, in: Marcus and Feldman's Osteoporosis, 5th ed., Academic Press, San Diego, CA, USA, 2021: pp. 405–452.

[4]. Knight R, Callewaert C, Marotz C, Hyde ER, Debelius JW, McDonald D, Sogin ML, The Microbiome and Human Biology, Annu Rev Genomics Hum Genet. 18 (2017) 65–86. 10.1146/annurev-genom-083115-022438. [PubMed: 28375652]

[5]. Gilbert JA, Blaser MJ, Caporaso JG, Jansson JK, Lynch SV, Knight R, Current understanding of the human microbiome, Nat Med. 24 (2018) 392–400. 10.1038/nm.4517. [PubMed: 29634682]

[6]. Lozupone CA, Stombaugh JI, Gordon JI, Jansson JK, Knight R, Diversity, stability and resilience of the human gut microbiota, Nature. 489 (2012) 220–30. 10.1038/nature11550. [PubMed: 22972295]

[7]. Koenig JE, Spor A, Scalfone N, Fricker AD, Stombaugh J, Knight R, Angenent LT, Ley RE, Succession of microbial consortia in the developing infant gut microbiome, Proc Natl Acad Sci U S A. 108 Suppl 1 (2011) 4578–85. 10.1073/pnas.1000081107. [PubMed: 20668239]

[8]. Yatsunenko T, Rey FE, Manary MJ, Trehan I, Dominguez-Bello MG, Contreras M, Magris M, Hidalgo G, Baldassano RN, Anokhin AP, Heath AC, Warner B, Reeder J, Kuczynski J, Caporaso JG, Lozupone CA, Lauber C, Clemente JC, Knights D, Knight R, Gordon JI, Human gut microbiome viewed across age and geography, Nature. 486 (2012) 222–7. 10.1038/nature11053. [PubMed: 22699611]

[9]. Bäckhed F, Roswall J, Peng Y, Feng Q, Jia H, Kovatcheva-Datchary P, Li Y, Xia Y, Xie H, Zhong H, Khan MT, Zhang J, Li J, Xiao L, Al-Aama J, Zhang D, Lee YS, Kotowska D, Colding C, Tremaroli V, Yin Y, Bergman S, Xu X, Madsen L, Kristiansen K, Dahlgren J, Wang J, Jun W, Dynamics and Stabilization of the Human Gut Microbiome during the First Year of Life, Cell Host Microbe. 17 (2015) 690–703. 10.1016/j.chom.2015.04.004. [PubMed: 25974306]

[10]. Brito IL, Gurry T, Zhao S, Huang K, Young SK, Shea TP, Naisilisili W, Jenkins AP, Jupiter SD, Gevers D, Alm EJ, Transmission of human-associated microbiota along family and social networks, Nat Microbiol. 4 (2019) 964–971. 10.1038/s41564-019-0409-6. [PubMed: 30911128]

[11]. David LA, Maurice CF, Carmody RN, Gootenberg DB, Button JE, Wolfe BE, Ling AV, Devlin AS, Varma Y, Fischbach MA, Biddinger SB, Dutton RJ, Turnbaugh PJ, Diet rapidly

and reproducibly alters the human gut microbiome, Nature. 505 (2014) 559–563. 10.1038/nature12820. [PubMed: 24336217]

[12]. Carmody RN, Turnbaugh PJ, Host-microbial interactions in the metabolism of therapeutic and diet-derived xenobiotics, J. Clin. Invest. 124 (2014) 4173–4181. 10.1172/JCI72335. [PubMed: 25105361]

[13]. Costello EK, Stagaman K, Dethlefsen L, Bohannan BJM, Relman DA, The application of ecological theory toward an understanding of the human microbiome, Science. 336 (2012) 1255–1262. 10.1126/science.1224203. [PubMed: 22674335]

[14]. McFall-Ngai M, Hadfield MG, Bosch TCG, Carey HV, Domazet-Lošo T, Douglas AE, Dubilier N, Eberl G, Fukami T, Gilbert SF, Hentschel U, King N, Kjelleberg S, Knoll AH, Kremer N, Mazmanian SK, Metcalf JL, Nealson K, Pierce NE, Rawls JF, Reid A, Ruby EG, Rumpho M, Sanders JG, Tautz D, Wernegreen JJ, Animals in a bacterial world, a new imperative for the life sciences, Proc Natl Acad Sci U S A. 110 (2013) 3229–3236. 10.1073/pnas.1218525110. [PubMed: 23391737]

[15]. Ley RE, Lozupone CA, Hamady M, Knight R, Gordon JI, Worlds within worlds: evolution of the vertebrate gut microbiota, Nat Rev Microbiol. 6 (2008) 776–788. 10.1038/nrmicro1978. [PubMed: 18794915]

[16]. Roth O, Beemelmanns A, Barribeau SM, Sadd BM, Recent advances in vertebrate and invertebrate transgenerational immunity in the light of ecology and evolution, Heredity (Edinb). 121 (2018) 225–238. 10.1038/s41437-018-0101-2. [PubMed: 29915335]

[17]. Austin B, Zhang X-H, Vibrio harveyi: a significant pathogen of marine vertebrates and invertebrates, Lett Appl Microbiol. 43 (2006) 119–124. 10.1111/j.1472-765X.2006.01989.x. [PubMed: 16869892]

[18]. Cooper MD, Alder MN, The evolution of adaptive immune systems, Cell. 124 (2006) 815–822. 10.1016/j.cell.2006.02.001. [PubMed: 16497590]

[19]. Adrian J, Bonsignore P, Hammer S, Frickey T, Hauck CR, Adaptation to Host-Specific Bacterial Pathogens Drives Rapid Evolution of a Human Innate Immune Receptor, Curr Biol. 29 (2019) 616-630.e5. 10.1016/j.cub.2019.01.058. [PubMed: 30744974]

[20]. Bates JM, Mittge E, Kuhlman J, Baden KN, Cheesman SE, Guillemin K, Distinct signals from the microbiota promote different aspects of zebrafish gut differentiation, Dev Biol. 297 (2006) 374–386. 10.1016/j.ydbio.2006.05.006. [PubMed: 16781702]

[21]. Stappenbeck TS, Hooper LV, Gordon JI, Developmental regulation of intestinal angiogenesis by indigenous microbes via Paneth cells, Proc Natl Acad Sci U S A. 99 (2002) 15451–15455. 10.1073/pnas.202604299. [PubMed: 12432102]

[22]. Chung H, Pamp SJ, Hill JA, Surana NK, Edelman SM, Troy EB, Reading NC, Villablanca EJ, Wang S, Mora JR, Umesaki Y, Mathis D, Benoist C, Relman DA, Kasper DL, Gut immune maturation depends on colonization with a host-specific microbiota, Cell. 149 (2012) 1578–1593. 10.1016/j.cell.2012.04.037. [PubMed: 22726443]

[23]. Needham BD, Kaddurah-Daouk R, Mazmanian SK, Gut microbial molecules in behavioural and neurodegenerative conditions, Nat Rev Neurosci. 21 (2020) 717–731. 10.1038/s41583-020-00381-0. [PubMed: 33067567]

[24]. Moeller AH, Suzuki TA, Lin D, Lacey EA, Wasser SK, Nachman MW, Dispersal limitation promotes the diversification of the mammalian gut microbiota, Proc Natl Acad Sci U S A. 114 (2017) 13768–13773. 10.1073/pnas.1700122114. [PubMed: 29229828]

[25]. Sepulveda J, Moeller AH, The Effects of Temperature on Animal Gut Microbiomes, Front Microbiol. 11 (2020) 384. 10.3389/fmicb.2020.00384. [PubMed: 32210948]

[26]. Song SJ, Sanders JG, Delsuc F, Metcalf J, Amato K, Taylor MW, Mazel F, Lutz HL, Winker K, Graves GR, Humphrey G, Gilbert JA, Hackett SJ, White KP, Skeen HR, Kurtis SM, Withrow J, Braile T, Miller M, McCracken KG, Maley JM, Ezenwa VO, Williams A, Blanton JM, McKenzie VJ, Knight R, Comparative Analyses of Vertebrate Gut Microbiomes Reveal Convergence between Birds and Bats, MBio. 11 (2020). 10.1128/mBio.02901-19.

[27]. Brooks AW, Kohl KD, Brucker RM, van Opstal EJ, Bordenstein SR, Phylosymbiosis: Relationships and Functional Effects of Microbial Communities across Host Evolutionary History, PLoS Biol. 14 (2016) e2000225. 10.1371/journal.pbio.2000225. [PubMed: 27861590]

[28]. Ochman H, Worobey M, Kuo C-H, Ndjango J-BN, Peeters M, Hahn BH, Hugenholtz P, Evolutionary relationships of wild hominids recapitulated by gut microbial communities, PLoS Biol. 8 (2010) e1000546. 10.1371/journal.pbio.1000546. [PubMed: 21103409]

[29]. Moeller AH, Li Y, Mpoudi Ngole E, Ahuka-Mundeke S, Lonsdorf EV, Pusey AE, Peeters M, Hahn BH, Ochman H, Rapid changes in the gut microbiome during human evolution, Proc Natl Acad Sci U S A. 111 (2014) 16431–16435. 10.1073/pnas.1419136111. [PubMed: 25368157]

[30]. Duranti S, Lugli GA, Milani C, James K, Mancabelli L, Turroni F, Alessandri G, Mangifesta M, Mancino W, Ossiprandi MC, Iori A, Rota C, Gargano G, Bernasconi S, Di Pierro F, van Sinderen D, Ventura M, Bifidobacterium bifidum and the infant gut microbiota: an intriguing case of microbe-host co-evolution, Environ Microbiol. 21 (2019) 3683–3695. 10.1111/1462-2920.14705. [PubMed: 31172651]

[31]. Moeller AH, Caro-Quintero A, Mjungu D, Georgiev AV, Lonsdorf EV, Muller MN, Pusey AE, Peeters M, Hahn BH, Ochman H, Cospeciation of gut microbiota with hominids, Science. 353 (2016) 380–2. 10.1126/science.aaf3951. [PubMed: 27463672]

[32]. Groussin M, Mazel F, Sanders JG, Smillie CS, Lavergne S, Thuiller W, Alm EJ, Unraveling the processes shaping mammalian gut microbiomes over evolutionary time, Nat Commun. 8 (2017) 14319. 10.1038/ncomms14319. [PubMed: 28230052]

[33]. Groussin M, Mazel F, Alm EJ, Co-evolution and Co-speciation of Host-Gut Bacteria Systems, Cell Host Microbe. 28 (2020) 12–22. 10.1016/j.chom.2020.06.013. [PubMed: 32645351]

[34]. Moeller AH, Gomes-Neto JC, Mantz S, Kittana H, Segura Munoz RR, Schmaltz RJ, Ramer-Tait AE, Nachman MW, Experimental Evidence for Adaptation to Species-Specific Gut Microbiota in House Mice, MSphere. 4 (2019). 10.1128/mSphere.00387-19.

[35]. Jukes TH, Williams WL, Nutritional effects of antibiotics, Pharmacol Rev. 5 (1953) 381–420. [PubMed: 13120335]

[36]. Ross E, Yacowitz H, Effect of Penicillin on Growth and Bone Ash of Chicks Fed Different Levels of Vitamin-D and Phosphorus, Poultry Sci. 33 (1954) 262–265.

[37]. Rusoff LL, Fussell JM, Hyde CE, Crown RM, Gall LS, Parenteral Administration of Aureomycin to Young Calves with a Note on Mode of Action, J Dairy Sci. 37 (1954) 488–497.

[38]. Cho I, Yamanishi S, Cox L, Methe BA, Zavadil J, Li K, Gao Z, Mahana D, Raju K, Teitler I, Li H, Alekseyenko AV, Blaser MJ, Antibiotics in early life alter the murine colonic microbiome and adiposity, Nature. 488 (2012) 621–6. 10.1038/nature11400. [PubMed: 22914093]

[39]. Cox LM, Yamanishi S, Sohn J, Alekseyenko AV, Leung JM, Cho I, Kim SG, Li H, Gao Z, Mahana D, Zarate Rodriguez JG, Rogers AB, Robine N, Loke P, Blaser MJ, Altering the intestinal microbiota during a critical developmental window has lasting metabolic consequences, Cell. 158 (2014) 705–21. 10.1016/j.cell.2014.05.052. [PubMed: 25126780]

[40]. Sjogren K, Engdahl C, Henning P, Lerner UH, Tremaroli V, Lagerquist MK, Backhed F, Ohlsson C, The gut microbiota regulates bone mass in mice, J Bone Miner Res. 27 (2012) 1357–67. 10.1002/jbmr.1588. [PubMed: 22407806]

[41]. Yan J, Herzog JW, Tsang K, Brennan CA, Bower MA, Garrett WS, Sartor BR, Aliprantis AO, Charles JF, Gut microbiota induce IGF-1 and promote bone formation and growth, Proc Natl Acad Sci U S A. 113 (2016) E7554–E7563. 10.1073/pnas.1607235113. [PubMed: 27821775]

[42]. Novince CM, Whittow CR, Aartun JD, Hathaway JD, Poulides N, Chavez MB, Steinkamp HM, Kirkwood KA, Huang E, Westwater C, Kirkwood KL, Commensal Gut Microbiota Immunomodulatory Actions in Bone Marrow and Liver have Catabolic Effects on Skeletal Homeostasis in Health, Sci Rep. 7 (2017) 5747. 10.1038/s41598-017-06126-x. [PubMed: 28720797]

[43]. Li JY, Chassaing B, Tyagi AM, Vaccaro C, Luo T, Adams J, Darby TM, Weitzmann MN, Mulle JG, Gewirtz AT, Jones RM, Pacifici R, Sex steroid deficiency-associated bone loss is microbiota dependent and prevented by probiotics, J. Clin. Invest. 126 (2016) 2049–2063. 10.1172/JCI86062. [PubMed: 27111232]

[44]. Li J-Y, Yu M, Pal S, Tyagi AM, Dar H, Adams J, Weitzmann MN, Jones RM, Pacifici R, Parathyroid hormone–dependent bone formation requires butyrate production by intestinal microbiota, Journal of Clinical Investigation. 130 (2020) 1767–1781. 10.1172/JCI133473.

[45]. Yu M, Malik Tyagi A, Li J-Y, Adams J, Denning TL, Weitzmann MN, Jones RM, Pacifici R, PTH induces bone loss via microbial-dependent expansion of intestinal TNF+ T cells and Th17 cells, Nat Commun. 11 (2020) 468. 10.1038/s41467-019-14148-4. [PubMed: 31980603]

[46]. Guss JD, Horsfield MW, Fontenele FF, Sandoval TN, Luna M, Apoorva F, Lima SF, Bicalho RC, Singh A, Ley RE, van der Meulen MC, Goldring SR, Hernandez CJ, Alterations to the Gut Microbiome Impair Bone Strength and Tissue Material Properties, J. Bone Miner. Res. 32 (2017) 1343–1353. 10.1002/jbmr.3114. [PubMed: 28244143]

[47]. Luna M, Guss JD, Vasquez-Bolanos LS, Castaneda M, Vargas Rojas M, Strong J, Dornevil S, Taylor EA, Bicalho RC, Donnelly EL, Hernandez CJ, Identifying components of the Gut Microbiome that Regulate Bone Tissue Mechanical Properties, in: Seattle, WA, USA, 2020: p. P-039.

[48]. Jepsen KJ, Silva MJ, Vashishth D, Guo XE, van der Meulen MC, Establishing biomechanical mechanisms in mouse models: practical guidelines for systematically evaluating phenotypic changes in the diaphyses of long bones, J. Bone Miner. Res. 30 (2015) 951–66. 10.1002/jbmr.2539. [PubMed: 25917136]

[49]. Schwarzer M, Makki K, Storelli G, Machuca-Gayet I, Srutkova D, Hermanova P, Martino ME, Balmand S, Hudcovic T, Heddi A, Rieusset J, Kozakova H, Vidal H, Leulier F, Lactobacillus plantarum strain maintains growth of infant mice during chronic undernutrition, Science. 351 (2016) 854–7. 10.1126/science.aad8588. [PubMed: 26912894]

[50]. O'Connor A, Quizon PM, Albright JE, Lin FT, Bennett BJ, Responsiveness of cardiometabolic-related microbiota to diet is influenced by host genetics, Mamm Genome. 25 (2014) 583–99. 10.1007/s00335-014-9540-0. [PubMed: 25159725]

[51]. Tyagi AM, Darby TM, Hsu E, Yu M, Pal S, Dar H, Li J-Y, Adams J, Jones RM, Pacifici R, The gut microbiota is a transmissible determinant of skeletal maturation, Elife. 10 (2021). 10.7554/eLife.64237.

[52]. Hernandez CJ, The Microbiome and Bone and Joint Disease, Curr Rheumatol Rep. 19 (2017) 77. 10.1007/s11926-017-0705-1. [PubMed: 29101487]

[53]. Pacifici R, Bone Remodeling and the Microbiome, Cold Spring Harb Perspect Med. 8 (2018). 10.1101/cshperspect.a031203.

[54]. Guss JD, Taylor E, Rouse Z, Roubert S, Higgins CH, Thomas CJ, Baker SP, Vashishth D, Donnelly E, Shea MK, Booth SL, Bicalho RC, Hernandez CJ, The microbial metagenome and bone tissue composition in mice with microbiome-induced reductions in bone strength, Bone. 127 (2019) 146–154. 10.1016/j.bone.2019.06.010. [PubMed: 31207357]

[55]. Hernandez CJ, Guss JD, Luna M, Goldring SR, Links Between the Microbiome and Bone, J Bone Miner Res. 31 (2016) 1638–1646. 10.1002/jbmr.2887. [PubMed: 27317164]

[56]. Guss JD, Ziemian SN, Luna M, Sandoval TN, Holyoak DT, Guisado GG, Roubert S, Callahan RL, Brito IL, van der Meulen MCH, Goldring SR, Hernandez CJ, The effects of metabolic syndrome, obesity, and the gut microbiome on load-induced osteoarthritis, Osteoarthritis Cartilage. 27 (2019) 129–139. 10.1016/j.joca.2018.07.020. [PubMed: 30240938]

[57]. Lam YM, Pearson OM, Bone density studies and the interpretation of the faunal record, Evol Anthropol. 14 (2005) 99–108. 10.1002/evan.20053.

[58]. Marín-Moratalla N, Jordana X, Köhler M, Bone histology as an approach to providing data on certain key life history traits in mammals: Implications for conservation biology, Mamm Biol. 78 (2013) 422–429. 10.1016/j.mambio.2013.07.079.

Table 1.

Summary of studies to date examining the role of the gut microbiome on bone.

Study	Animal Age (manipulation	Cancellous Bone	Cortical Bone
Sjogren et al. 2012 [40]	2 month old mice (germ-free v. conventional)	Increased in Germ-free mice	Increased in Germ-free mice
Cho et al. 2012 [38]	< 2 month old mice	Oral antibiotics influence BMD in young but not older mice	
Schwarzer et al. 2016 [49]	2 month old mice (germ-free v. conventional)	Reduced in Germ-free	Reduced in Germ-free
Cox et. al. 2014 [39]	5 month old mice	Oral antibiotics influence BMD in young but not older mice	
Yan et al. 2016 [41]	3 months old (germ-free v. conventionalized at 2 months of age)	Increased BV/TV in Germ-free mice or following decimation of the gut microbiota with antibiotics	NR
Novince et al. 2017[42]	3 months of age (germ-free v. specific pathogen free)	Increased BV/TV in Germ-free mice (n=4/group)	No differences in cortical geometry observed.
Li et al. 2020 [44]	3 months of age(germ-free v. conventional)	No differences in trabecular BV/TV	Germ-free increased cortical thickness but not cortical area
Li et al. 2020 [44]	3 months of age (untreated v. decimation of gut microbiota starting at 1.5 months of age)	No differences in trabecular BV/TV	Cortical area and thickness reduced with decimation of the gut microbiota
Guss et al. 2017 [46]	4 months of age (untreated v. disruption of microbiota starting at 1 month of age)	No differences in trabecular BV/TV	Disruption of gut microbiota causes small reduction in femur length; no differences in cortical geometry
Luna et al. 2021 [47]	4 months of age (untreated v. disruption of microbiota starting at 1 month of age)	No differences in trabecular BV/TV	Disruption of gut microbiota caused small reductions in femur length; reductions in measures of cortical geometry
Yu et. al. 2020 [45]	4.5 months of age(untreated v. decimation of gut microbiota starting at 3.5 months of age)	No differences in trabecular BV/TV	No differences in measures of cortical geometry
Li et al. 2016 [43]	5 months of age (germ-free v. conventionalized at 1 month)	No differences Germ-free v. Conventional	Increased cortical cross-sectional area in Germ-free mice
Yan et al. 2016 [41]	10 months old (germ-free v. conventionalized at 2 months of age)	No difference Germ-free v. conventionalized mice	Germ-free mice have reduced cortical geometry