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REVIEW

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Gut microbiota as a target in the bone health of livestock and poultry: roles of short-chain fatty acids

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Abstract

The regulation and maintenance of bone metabolic homeostasis are crucial for animal skeletal health. It has been established that structural alterations in the gut microbiota and ecological dysbiosis are closely associated with bone metabolic homeostasis. The gut microbiota and its metabolites, especially short-chain fatty acids (SCFAs), affect almost all organs, including the bone. In this process, SCFAs positively affect bone healing by acting directly on cells involved in bone repair after or by shaping appropriate anti-inflammatory and immunomodulatory responses. Additionally, SCFAs have the potential to maintain bone health in livestock and poultry because of their various biological functions in regulating bone metabolism, including immune function, calcium absorption, osteogenesis and osteolysis. This review primarily focuses on the role of SCFAs in the regulation of bone metabolism by gut microbiota and provides insight into studies related to bone health in livestock and poultry.

Keywords Bone disease, Bone metabolism, Gut microbiota, Probiotics, Short-chain fatty acids

Introduction

The skeleton, which is made up of bones, has a number of functions, including bearing the weight of the body, storing minerals like calcium and phosphorus, and producing blood cells in the bone marrow. Therefore, the management and upkeep of bone metabolic homeostasis are crucial for the health of an animal's skeleton. Bone metabolism is characterized by the close cooperation of bone cells (including osteoblasts, osteoclasts and osteocytes) to maintain the number and integrity of bone microarchitecture, and once the homeostasis of bone metabolism is disrupted, it may result in bone loss, greatly increasing the risk of bone diseases (Barsony et al. 2019). Bone disease, a phenomenon of prevalent occurrence in the modern poultry industry, results from the disruption of the normal processes of bone formation and homeostasis. It is estimated that the U.S. broiler industry lost $80–120 million annually due to leg disease in the early 1990s. This problem still poses more significant threat to the broiler industry with the increased intensive management of poultry today (Xu et al. 2022a), which not only results in motor dysfunction accompanied by symptoms like claudication, slow movement, and difficulty in standing (Xu et al. 2022b) but also decreases production performance and meat quality (Huang et al. 2021; Cao et al. 2020), leading in huge economic losses. In addition, animals including pigs, cattle, and sheep are also susceptible to bone illnesses, but there are currently no viable treatments to prevent or slow the progression of these conditions (Hejazi and Danyluk 2009; Tóth et al.

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2017; Yadav et al. 2019). As a result, the bone disease has extremely extensive impacts, making it essential to explore efficient remedies.

According to recent research, gut microbiota plays a critical regulatory role in maintaining bone homeostasis and bone health (Chen et al. 2022). Intestinal microbes can influence bone homeostasis by participating in metabolic, immune and endocrine processes (Xu et al. 2022a). In the gastrointestinal tract (GIT), gut microbiota and host cells interact in ways that are typically advantageous to the host, including promoting the maturation of the intestinal immune system through interaction with immune cells (e.g., macrophages and dendritic cells) and maintaining the integrity of the intestinal barrier by inducing mucus production and providing nutrition to the intestinal epithelial cells (Xu et al. 2022a). However, alterations in the gut microbiota can disrupt the beneficial microbe-host relationship, which leads to the development or progression of diseases, including inflammatory bowel disease, cardiovascular disease, asthma, and rheumatoid arthritis (Deal 2012; Xu et al. 2022a; Zhang et al. 2022).

Several studies have confirmed that the gut microbiota affects bone metabolic homeostasis through various pathways and that short-chain fatty acids (SCFAs), metabolites of the gut microbiota, play a key role in bone metabolism, including immunity, calcium absorption, deposition, osteogenesis and osteolysis (Holscher 2017; Sanders et al. 2019; Zhang et al. 2021; Xu et al. 2023). This paper summarizes recent publications on the regulation of bone metabolic homeostasis by SCFAs, aiming to explore and introduce the regulatory role of SCFAs in bone metabolism and to provide insight for the prevention and treatment of skeletal diseases in livestock and poultry.

Bone metabolism and gut microbiota

Bone metabolism occurs in a dynamic equilibrium between bone resorption and bone formation, and this process requires the synergistic action of osteoclasts and osteoblasts (Xu et al. 2022b). Different roles are played by osteoblasts and osteoclasts in maintaining animals' appropriate amounts of bone mass. In contrast to osteoclasts, which are primarily in charge of resorbing the bone matrix, osteoblasts are responsible for the synthesis, secretion and mineralization of bone matrix (Hu and Olsen 2016; Ono and Nakashima 2018). In addition, the synergistic effect of osteoblasts and osteoclasts is influenced by several factors, such as stem cell antigens, hormones, growth factors, and SCFAs (Chen et al. 2018, 2022; Lucas et al. 2018).

Animal tissues and organs are inextricably linked to one another. The intestine, the largest immunological organ in both humans and animals, plays an important role in the immune system and the equilibrium of bone metabolism (Alonso et al. 2014). Peek et al. (2022) confirmed that intestinal inflammation strengthens the differentiation of osteoclasts, which significantly increases bone loss and jeopardizes bone health. Moreover, the microbes in the gut also have a key role in the regulation of bone mass. A study by Xi et al. (2022) demonstrated that favorable alterations in gut microecology under the influence of probiotics could alleviate bone loss caused by rheumatoid joints. The above findings show a strong correlation between gut and bone health.

In the GIT, microorganisms and their host have a complex, mutually beneficial symbiotic interaction resulting from their long-term coevolution and reciprocal influence. These huge and richly diversified microbial communities contribute significantly to the preservation of bone health by regulating bone metabolism through their metabolites or gut microbiota-mediated regulators of bone metabolism (Xu et al. 2022a). It has been demonstrated that the gut microbiota can regulate the ratio and relative activity of osteoclasts and osteoblasts through multiple pathways, thereby affecting bone metabolism and bone development (Behera et al. 2020). SCFAs, the metabolites of gut microbes, regulate osteoblasts' differentiation, proliferation, and apoptosis through regulatory T cells (Tregs), thereby affecting bone metabolic processes. Furthermore, Yan reported that SCFAs could regulate serum levels of insulin-like growth factor 1 and improve bone growth and health (Yan et al. 2016). Therefore, SCFAs may be a fundamental substance in controlling bone metabolism and preventing bone loss by the gut microbiota.

Bone health and SCFAs

Composition and origin of SCFAs

SCFAs, including a class of acid metabolites such as acetic acid, propionic acid, butyric acid, valeric acid, isobutyl and isovaleric acid, are produced by bacteria in the gut of humans and animals by the fermentation of indigestible carbohydrates and proteins in food (Sam et al. 2021). SCFAs are derived from the soluble dietary fiber found in foods like oligosaccharides (bananas, onions and asparagus), pectin (apples, apricots, carrots, oranges), kidney beans, oat bran, corn starch, milk, yogurt, and sprouted barley. Other significant sources of SCFAs include resistant starches including barley, rice, beans, green bananas, and potatoes (Barber et al. 2020; Li et al. 2020; P and Joye 2020; Cronin et al. 2021). These indigestible fibers are not digested and absorbed in the small intestine and are subsequently fermented by microbiota in the

cecum and large intestine; SCFAs are mostly generated in the cecum as well as the proximal colon and less in the distal colon (Sun et al. 2017a, b). The primary constituents of intestinal SCFAs are acetic acid, propionic acid, and butyric acid, which together make for 90%-95% of the total amount of SCFAs generated by gut microbiota (Wong et al. 2006). Therefore, the three types of SCFAs described above have been studied extensively, particularly butyric acid.

Biological functions of SCFAs

SCFAs are closely related to intestinal microecology and are involved in a wide range of biological processes, including signal transduction, cytokine modulation, immune cell regulation and intestinal mucosal barrier function (Tan et al. 2014; Louis and Flint 2017). Furthermore, it has been confirmed that SCFAs increase bone formation and improve bone quality by regulating immunity, intestinal barrier function and immune cell activity (Wong et al. 2006; Li et al. 2020; Xu et al. 2022a).

At the intestinal endothelium barrier, host cells can directly transport SCFAs throughout the body, thus affecting distant tissues. GPR43, GPR41 and GPR109a are examples of G protein-coupled receptors (GPRs) that may lind to SCFAs (Zaiss et al. 2019). These membrane-bound receptors are expressed on various immune cell types, including monocyte macrophages, as well as on other non-immune cells, such as intestinal epithelial cells, adipocytes and enteroendocrine cells (Sun et al. 2017a, b). According to Melhem et al. (Melhem et al. 2019), the binding of these receptors to SCFA leads to the release of intracellular Ca^{2+} and the activation of different downstream signaling pathways, including the Extracellular signal-regulated kinase/mitogen-activated protein kinase, p38 or Phosphatidylinositide 3-kinases signaling pathway, which regulates cellular activity and function. Additionally, some SCFAs, like butyrate, serve as a major source of energy for the essential functions of intestinal epithelial cells, which directly affect the formation and proliferation of these cells. Nevertheless, a certain number of SCFAs reach the bloodstream through transport systems (monocarboxylate transporter 1/Solute Carrier family 16 member 1 pathway or passive diffusion) and are transported to the whole body through the transport system. Once enter the circulatory system, SCFAs affect the metabolism and function of peripheral tissues (adipose tissue, skeletal muscle, bone, etc.) by activating GPRs (Zhou and Fan 2019; Li et al. 2021).

Butyric acid and bone health

Butyric acid ($C_4H_8O_2$) exhibits the most extensive biological activity among SCFAs, including regulating inflammation, maintaining immune homeostasis, and lessening bone loss caused by inflammation. By inhibiting GPR41 and HDAC, butyric acid can increase the production of IL-22, while downregulate the proinflammatory mediators NO, interleukin-6 (IL-6) and IL-12 produced by lipopolysaccharide (LPS)-induced macrophages (Nastasi et al. 2017). Butyric acid inhibits the maturation and biological activity of monocyte-derived dendritic cells and promotes the polarization of early CD4+T cells into IL-10-producing Tregs that are stimulated by LPS (Park et al. 2016). Butyric acid regulates the inflammatory state of the body by activating GPRs in the intestinal mucosal epithelium, reducing the synthesis and secretion of proinflammatory factors such as tumor necrosis factor-α (TNF-α) and cyclooxygenase-2, thereby reducing the bone loss caused by inflammation (Yang et al. 2020; Zhang et al. 2022).

Furthermore, butyric acid alleviates intestinal inflammation and reduces osteoclast differentiation by attenuating TNF-α-mediated immune responses and reducing inflammatory vesicles such as NOD-like receptor protein 3 (NLRP3) (Clark and Mach 2017). Additionally, butyrate has been reported to control the expression of claudin-2, lower intestinal permeability via an IL-10 receptor-dependent mechanism, and strengthen intestinal barrier function by increasing colonic mucin and tight junction protein production (Barsony et al. 2019; Gonzalez et al. 2019), enhancing immune response system function and thereby prevent bone loss (Yan and Ajuwon 2017). Kaisar et al. (2017) found that butyrate suppressed the expression of the proinflammatory factor Interferon-γ (IFN-γ) caused by overactivation of the IFN-γ/signal transducer and activator of transcription 1 (STAT1) signaling pathway. Moreover, butyrate also modulates the immune effect and relieves osteoarthritis by inhibiting the activity of the inflammation-related pathways Nuclear factor kappa-B (NF-κB), Janus Kinase /STAT, IL-12p70, and IL-23 and preventing the polarization of early CD4+T lymphocytes into T helper cells 1 (Th1) and Th17 cells (Dalile et al. 2019). In summary, butyric acid helps to maintain bone health by regulating immune function and alleviating bone destruction and loss.

Acetic acid and bone health

Acetic acid ($C_2H_4O_2$) is one of the most abundant SCFAs produced by the gut microbiota (Lavelle and Sokol 2020). It positively impacts bone health by preserving the integrity of the intestinal mucosal barrier, limiting the invasion of pathogenic bacteria and enhancing the host's immune function. According to Deleu , acetic acid is mostly found in tissues, excrement, and blood in animals (Deleu et al. 2021). Acetic acid increases the production of IgA in the colon, alters the ability of IgA to bind to specific intestinal bacteria, and alters the colonization of these bacteria,

enhancing the immune barrier function of the intestinal mucosa, thereby indirectly reducing the release of proinflammatory factors like TNF-α and IL-1β, inhibiting osteoclast activity and preventing bone loss (Boets et al. 2017; Takeuchi et al. 2021). In addition, acetic acid can activate the GPR41 receptor on the surface of immune cells, enhancing the immune effect and facilitating the maintenance of bone health (Le Poul et al. 2003; Kobayashi et al. 2016). Maslowski demonstrated that acetic acid significantly enhanced intestinal function and reduced DNA-dependent activator of interferon-regulatory factors and inflammatory mediator myeloperoxidase levels and TNF-α, thereby facilitating the remission of the inflammatory response and reducing osteoclast production and differentiation (Maslowski et al. 2009). According to the aforementioned information, acetic acid enhances immune function and inhibits the release of proinflammatory factors, which prevents the activation of osteolytic effects.

Propionic acid and bone health

Propionic acid ($C_3H_6O_2$) is an organic acid that naturally develops as a result of the kind of bacterial action found on the skin or in the GIT. After entering the circulation, propionic acid in the gut performs a variety of functions, including affecting hepatic cholesterol metabolism, promoting calcium absorption, increasing calcium deposition, and facilitating bone formation (Hirschberg et al. 2019; Lavelle and Sokol 2020). In addition, propionic acid not only activates NLRP3 inflammatory vesicles in intestinal epithelial cells, induces IL-18 secretion, and improves the integrity of the intestinal mucosal epithelial barrier, but also inhibits histone deacetylase and lowers NF-κB activity, thereby reducing the release of the inflammatory factors TNF-α, IL-6, and IL-8 and affecting the structure and function of the intestinal mucosal barrier (Duscha et al. 2020). It has been demonstrated that osteoporosis is closely associated with the cellular imbalance of the immune system and immune-mediated effects on bone formation through the gut (Arpaia et al. 2013). Interestingly, mice with SCFA intake exhibited increased bone mass in mice accompanied by a decrease in inflammation-induced bone loss. In a study on the effects of propionic acid supplementation on human bone metabolism, Duscha et al. (2022) uncovered that intake of propionic acid significantly increased serum levels of osteocalcin (a marker of bone formation), and decreased β-CrossLaps levels (a marker of bone resorption), suggesting that propionic acid intake increases bone formation and decreases bone resorption.

Valeric acid and bone health

The GIT had high levels of propionate and butyrate and low levels of valeric acid ($C_5H_{10}O_2$) (Cummings et al. 1987). However, a study confirmed that total specific inhibition of HDAC and promotion of differentiation of Tregs into T cells by promoting the capacity of intestinal microorganisms to generate butyric and valeric acid could enhance bone immunity indirectly (Yuille et al. 2018). Studies have demonstrated that valeric acid levels are initially low in animals, and dietary fiber intake helps to increase valeric acid levels, which contributes to reducing the release of proinflammatory factors and mitigates bone destruction (Yuille et al. 2018; Gio-Batta et al. 2022), indicating the immunomodulatory ability of valeric acid and its potential therapeutic value for inflammation-induced bone diseases.

There are similarities in the functions of the different SCFAs. For example, they can regulate the composition of the gut microbiota by balancing gut pH and preventing the colonization of pathogenic bacteria, which facilitates the establishment of the intestinal immune barrier and inhibits the release of inflammation-related signaling molecules IL-6, IL-7, receptor activator for nuclear factor-κB ligand (RANKL), thereby reduces osteoclast differentiation and promotes bone health. According to the aforementioned data, a close association between bone health and gut health is established through SCFAs.

Gut microbiota and SCFAs

Different types of gut microbes generate different amounts of SCFAs through fermentation (Table 1). The study of Wolin confirmed that the fermentation products of *Lactobacillus* are mainly lactic acids (Wolin et al. 1999). The fermentation products of *Megasphaera elsdenii* are mainly acetic acid and butyric acid, whereas the fermentation products of *Bifidobacterium* are mostly acetic acid, lactic acid, and formic acid. It can be seen that different types of gut microbes produce different SCFAs. In addition, dietary fiber is important for the composition and metabolic function of the gut microbiota and can affect the amount of SCFAs. Diet has a decisive role in the composition of the gut microbiota and the amount of SCFAs. Therefore, it is possible to alter the structure of the gut microbiota through diet to regulate SCFAs. Even short-term dietary interventions can have a significant impact on gut microbiota structure. In particular, diets based exclusively on animal products, consuming reduced-fat foods high in protein and low in carbohydrates or fiber, can cause an imbalance in the structure of the microorganism by increasing the relative abundance of Bacteroidetes and decreasing the relative quantity of Firmicutes, while affecting SCFA concentrations in the intestine (Simpson and Campbell 2015). In conclusion, long-term poor dietary habits may increase the risk of intestinal

Table 1 Gut microbiota for the synthesis of SCFAs

Phylum	Family	Genus/Species	SCFAs	References
Actinobacteria	Bifidobacteriaceae	*Bifidobacterium adolescentis*	Propionic acid	Reichardt et al. 2014
		Bifidobacterium spp.	Acetic acid	
Bacteroidetes	Bacteroidaceae	*Bacteroides fragilis*	Propionic acid Butyric acid	Scott et al. 2006; Reichardt et al. 2014; Tang et al. 2019
		Bacteroides spp.	Acetic acid Propionic acid	
		Bacteroides thetaiotaomicron	Propionic acid Butyric acid	
	Prevotellaceae	*Prevotella stercorea*	Acetic acid Valeric acid	Hayashi et al. 2007
Firmicutes	Streptococcaceae	*Streptococcus spp.*	Acetic acid	Scott et al. 2006; Louis et al. 2014
	Clostridiaceae	*Clostridium beijerickii*	Propionic acid Butyric acid	Scott et al. 2006; Rey et al. 2010; Shetty et al. 2013; Van et al. 2013; Louis et al. 2014; Reichardt et al. 2014
		Clostridium botulinum	Propionic acid Butyric acid	
		Clostridium butyricum	Butyric acid	
	Lachnospiraceae	*Coprococcus comes*	Propionic acid Butyric acid	
		Coprococcus eutactus	Propionic acid Butyric acid	
	Veillonellaceae	*Megasphaera elsdenii*	Acetic acid Propionic acid Butyric acid Valeric acid	Shetty et al. 2013; Louis et al. 2014
		Megasphaera spp.	Acetic acid Propionic acid Butyric acid Valeric acid	
Proteobacteria	Enterobacteriaceae	*Salmonella spp.*	Propionic acid	Shetty et al. 2013
Verrucomicrobia	Verrucomicrobiaceae	*Akkermansia muciniphila*	Acetic acid Propionic acid	Van et al. 2013

Only part of the gut microbiota that can produce SCFA is listed in the table

diseases, and the intake of more fermentable dietary fiber can regulate the composition of the gut microbiota and thus positively affect the prevention of diseases.

When investigating the concentration of SCFAs under different gut microbiota structures, it was found that the capacity of the gut microbiota to produce SCFAs was enhanced by the addition of certain probiotics (LeBlanc et al. 2017). For example, in a broiler cecum model, supplementation with *Lactobacillus* (*L.*) *salivarius* increased the concentrations of propionate and butyrate in the cecum (Meimandipour et al. 2010). Furthermore, it was summarized that changing the composition of gut microbiota by taking antibiotics, dietary changes and adding probiotics can affect bone health (Martin-Gallausiaux et al. 2021). These findings show that the structure of gut microbiota can be altered by diet, which further affects SCFAs concentration, and that different species of gut microbiota produce different types and amounts of SCFAs. Additionally, it implies that SCFAs produced by the gut microbiota may regulate bone metabolism.

Regulation of SCFAs on bone metabolism

As bioinformatics and molecular biotechnology have developed, increasing studies have demonstrated that gut microbiota metabolites regulate bone metabolism (Zaiss et al. 2019; He et al. 2020; Xu et al. 2023). Among metabolites in animals, SCFAs have been shown to affect almost all body organs, including bone (Roberfroid et al. 2010; Xu et al. 2023). SCFAs can participate in bone metabolism by directly acting on osteoblasts, osteoclasts, chondrocytes, and fibroblasts or indirectly by regulating the absorption of mineral elements and can also affect bone metabolism through modulating the immune system (Yan and Charles 2017; Yan et al. 2018) (Fig. 1). Thus, SCFAs exhibit a wide range of beneficial effects on bone quality enhancement and bone metabolism activities.

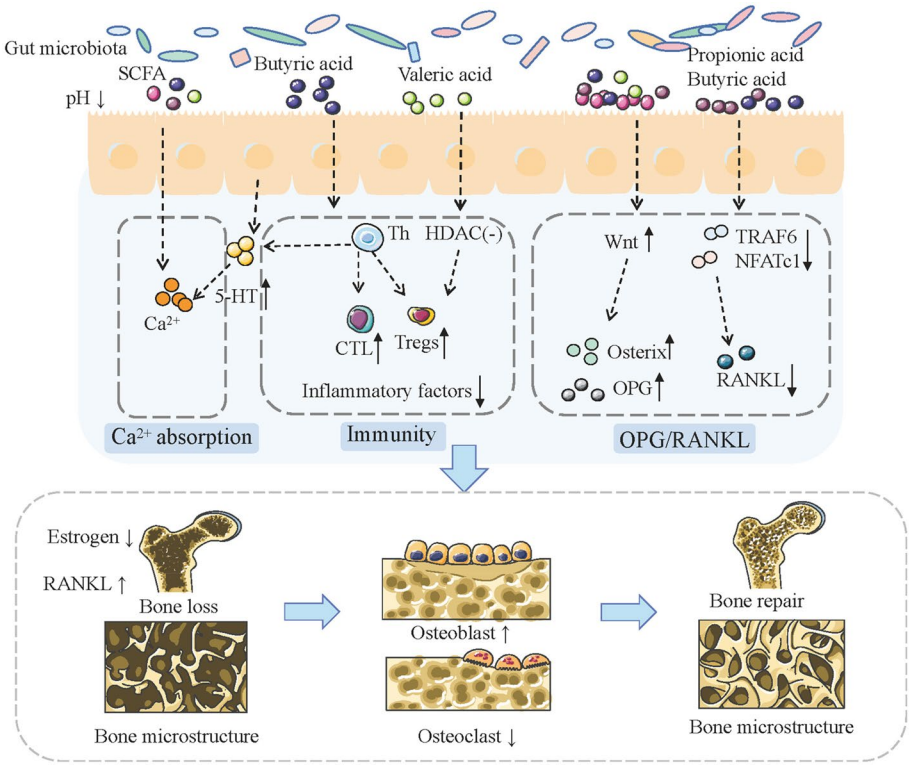

Fig. 1 SCFAs produced by gut microbiota facilitate bone health. SCFAs can reduce intestinal pH and promote calcium absorption. In terms of immunity, SCFAs induce Th differentiation into CTLs and Tregs and regulate serotonin content through Th, which directly or indirectly promotes bone formation and inhibits bone resorption. Furthermore, SCFAs regulate Tregs by inhibiting HDAC and activate osteogenic differentiation and osteoclast differentiation-related genes to control bone metabolism. SCFAs also promote the differentiation and activity of osteoblasts and inhibit the activity of osteoclasts to some extent, thus facilitating the calcification and deposition of bones and reducing the risk of bone loss (e.g., estrogen deficiency). Meanwhile, it can suppress the secretion of inflammatory mediators, which is beneficial to bone health

SCFAs regulate bone metabolism through the immune system

SCFAs promote the maturation of the animal immune system and regulate bone metabolism through the immune system (Morrison and Preston 2016). Studies have shown that gut microbiota can interact with immune cells and dendritic cells to promote the production of molecules like SCFAs, indole derivatives, polyamines and secondary bile acids (D'Amelio and Sassi 2018). In particular, immune cells express SCFA receptors, and the binding of SCFA to the corresponding receptors has a regulatory effect on T-cell function and immune cell differentiation, thereby enhancing immune function and preventing inflammation-related bone loss (Yuille et al. 2018; Gio-Batta et al. 2022). In addition, there is evidence that in osteoporosis caused by estrogen deficiency, T cells can increase the production of pro-inflammatory and pro-osteoclastic cytokines in bone tissue. Such as TNF-α and RANKL, and that the upregulation of the expression of these factors in osteoblasts enhances the osteoclastogenesis induced by Th17 to stimulate the regulation of bone resorption (D'Amelio et al. 2008). Therefore, SCFAs may play an important role in bone metabolism and bone mineral density due to their close relationship with immune cells and bone cells.

Furthermore, butyrate and propionate have been shown to regulate intestinal immunological function by inhibiting histone deacetylase (HDAC) (Ratajczak et al. 2019). Chang demonstrated that butyrate produced by gut microbiota acts as an inhibitor of HDAC and modulates the function of macrophages in the lamina propria of the mouse gut. Related studies have shown that the inhibition of HDAC increases the development and function of Tregs (Chang et al. 2014). Therefore, it may be one of the mechanisms by which SCFAs enhance the production of Tregs in the GIT (Huang et al. 2017). The interaction of SCFAs with GPRs not only drives the differentiation of T cells into Tregs (Fig. 1) but also promotes differentiation into effector T cells (Kim et al. 2013). Park and colleagues suggested that SCFAs may induce helper T cells to differentiate into Th1 and Th17, thereby increasing host resistance to pathogen attack (Trompette et al. 2014; Park et al. 2015). SCFAs such as butyrate and propionate also regulate antigen presentation through HDAC inhibition

by interacting with GPRs and have an inhibitory effect on dendritic cell development. Together, the gut microbiota can affect the overall immune function of the host through SCFAs, immune cells and immune factors acting on bone tissue to affect the biochemical activity of osteoblasts in this process and exert a regulatory effect on bone homeostasis and bone mass.

SCFAs affect calcium absorption, BMD and bone strength

SCFAs can increase calcium deposition, bone mineral density (BMD) and mechanical strength (Weaver 2015). As we all know, calcium is an essential element for bone formation, and sufficient calcium is necessary to improve bone quality and increase bone mechanical strength (Rovenský et al. 2003; Zhao et al. 2020a, b). To achieve a healthy peak bone mass and prevent the onset of bone loss, maintaining calcium balance is essential. Prebiotics are food components that selectively stimulate the growth or activity of one or several bacteria in the colon, thus exerting a beneficial effect on the host and helping to regulate the composition of the gut microbiota (Quigley 2019). The production of SCFAs by the fermentation of prebiotics by gut microbiota can enhance calcium absorption, bone mineral density, and bone strength (Weaver 2015). A study found that daily prebiotic fiber intake can enhance calcium absorption in adolescent children, which is beneficial to the bone development of adolescent-aged children (Whisner et al. 2014). SCFAs are key to the impact of prebiotic fiber on bone. Dietary fiber intake affects calcium absorption: increased levels of intestinal SCFAs after fermentation by gut microbiota can reduce the pH of the intestinal microecology, thus reducing the formation of calcium phosphate and increasing calcium production and absorption (Wallace et al. 2017). A study by Wallimann also confirmed that butyric acid significantly increases calcium deposition at the site of bone injury and promotes bone healing (Wallimann et al. 2021). The above demonstrates the potential of SFCAs in promoting skeletal growth.

The effect of SCFAs on calcium deposition may not only be reflected in changes in intestinal pH. Indeed, SCFAs have been shown to increase calcium transport by regulating some signaling pathways (Gultemirian et al. 2014; Chen et al. 2019). Furthermore, SCFAs can indirectly improve calcium absorption by modulating the production of intestinal serotonin, namely, 5-hydroxytryptamine (5-HT, Wang et al. 2020). Serotonin, a molecule that interacts with osteoblasts, has been used as a potential regulator of bone mass to prevent osteoporosis by increasing bone formation (De Vernejoul et al. 2012). Duodenal enterochromaffin cells have the biological function of synthesizing 5-HT and can promote the synthesis of 5-HT under the action of SCFAs (Ducy 2011). It has been reported that 5-HT can interact with osteoblasts, especially by activating the 5-HT1B receptor on preosteoblasts to reduce osteoblast proliferation and thus improve the bone loss caused by osteoporosis (Reigstad et al. 2015). In conclusion, SCFAs can directly or indirectly regulate bone formation, thereby increasing bone mineral density and bone strength and reducing fracture risk.

The regulatory role of SCFAs in osteoblasts

SCFAs can promote bone formation by regulating osteoblast activity. Osteoblasts have a pivotal role in bone formation, and the activation of Wnt signaling in osteoblasts is essential for osteoblast proliferation and bone homeostasis (Chen et al. 2019). Studies have demonstrated that the Wnt signaling pathway is crucial for bone growth and endostasis; moreover, SCFAs can activate the Wnt pathway and induce the expression of the transcription factor osterix, thereby promoting osteoblast differentiation (Fig. 1) (Kobayashi et al. 2016; Chen et al. 2019). In addition, this signaling pathway stimulates osteoprotegerin (OPG), an osteoclast suppressor, to be expressed in osteoblast lineage cells, which prevents bone resorption (Xu et al. 2022a, b). In a human study by Katono, it was found that butyrate can affect normal osteoblasts, increase osteoblast mineralization to promote bone formation and inhibit osteoclast differentiation by promoting OPG production (Katono et al. 2008).

SCFAs can indirectly regulate bone metabolism through Tregs. Tregs regulate the body's immune function by actively regulating the activation and proliferation of potentially self-reactive T cells in normal organisms. In addition to their immunomodulatory functions, Tregs can also exert some regulatory effects on bone homeostasis. They inhibit osteoclastogenesis, promote osteoblast differentiation and are required for parathyroid hormone-stimulated bone formation (Ko 2017). Tregs were isolated from SPF mice and cultured in vitro with SCFAs. It was found that in the presence of propionate, the proliferation of Tregs can be promoted, thereby enhancing the regulation of bone homeostasis by Tregs (Smith et al. 2013). Supplementation with probiotics can alleviate pathological bone loss to some extent. Our study has demonstrated that *L. rhamnosus* prevented thiram-induced tibial dyschondroplasia by improving bone-related growth performance in broilers, including tibia weight, length, and mean diameter (Liu et al. 2021). Tyagi found that supplementation of *L. rhamnosus* in mouse diets can affect bone homeostasis, and the results showed that *L. rhamnosus* increased the volume of bone trabeculae and promoted increased bone formation (Tyagi et al. 2018). This was attributed to the production of butyrate in the gut after *L. rhamnosus* ingestion, which induced

the proliferation of Tregs in the intestine and bone tissue. The same results were obtained in an experiment where butyrate was fed directly to germ-free mice (Doublier et al. 2022). These studies suggested that SCFAs may indirectly regulate bone homeostasis through the biological function of Tregs.

Table 2 The function and role of SCFAs involved in bone health

Function	Types of SCFA	Object of study	Effect	References
Immunity	Acetic acid Propionic acid Butyric acid Isobutyric acid Valeric acid Isovaleric acid	Mice	Probiotics can regulate the immune response and relieve inflammation by producing SCFAs	Khan et al. 2022
	Butyric acid	Mice	The development of arthritis in mice is inhibited by butyrate by modulating cellular and humoral immune responses, and it has an ameliorative effect on bone	He et al. 2022
	Acetic acid Butyric acid Isobutyric acid	Mice	The production of SCFAs has a mitigating effect on OP development	Zhao et al. 2020a, b
The regulation of calcium	Acetic acid Propionic acid Butyric acid	Laying hens	Promote intestinal absorption of calcium	Gultemirian et al. 2014
	Acetic acid Propionic acid Butyric acid Valeric acid	Mice	Increases calcium deposition at the site of bone injury and accelerates bone formation	Wallimann et al. 2021
	Acetic acid Propionic acid Butyric acid Isobutyric acid Valeric acid Isovaleric acid	Rats	Increases mineral availability by increasing calcium dissolution at lower pH, thereby increasing bone mineral content and deposition	Weaver et al. 2010
Osteogenesis	Butyric acid	Human beings	Increased mineralization of osteoblasts promotes bone formation and inhibits osteoclast differentiation by promoting OPG production by human osteoblasts	Ko. 2017
	Butyric acid	Mice	Butyrates can increase the number of Tregs in the intestine and bone marrow. Tregs can stimulate CD8 + T cells, which can secrete Wnt10b and promote bone formation by activating Wnt signaling in osteoblasts	Arpaia et al. 2013; Chen et al. 2019
	Propionic acid	SPF Mice	Propionate can promote the proliferation of Tregs, thereby enhancing the regulation of Tregs on bone homeostasis	Liu et al. 2021
	Butyric acid	Mice	The induced proliferation of Tregs in the gut and bone tissue and increased trabecular bone volume, and promoted bone formation in mice	Tyagi et al. 2018
Osteoclastogenesis	Butyric acid	Rats, Mice	Butyrate can inhibit the production of osteoclast precursor cells by inhibiting the activity of Histone deacetylase (HDAC). Butyric acid inhibits the formation of osteoclasts and the expression of osteoclast-specific mRNA under the stimulation of RANKL	Rahman et al. 2003
	Isovaleric acid	Mice	Isovaleric acid suppresses differentiation of bone marrow-derived macrophages into OCs by RANKL. Isovaleric acid inhibited the expression of OC-related genes	Cho et al. 2021
	Acetic acid Propionic acid Butyric acid Valeric acid	Mice	Genes related to osteoclast differentiation are differentially expressed in osteoclast precursor cells, which can significantly reduce osteoclast formation and bone resorption activity	Wallimann et al. 2021

Abbreviations: *OP* Osteoporosis, *HDAC* Histone deacetylase, *RANKL* Receptor activator of nuclear factor-κB ligand

The regulatory role of SCFAs in osteoclasts

SCFAs have the ability to modulate osteoclast activity, which in turn regulates bone resorption. Osteoclasts are critical cells involved in the regulation of bone resorption and are essential for maintaining the homeostasis of bone metabolism. Montalvany-Antonucci studied the effect of SCFA on alveolar bone and found that SCFA acts as a regulator of bone resorption and reduces osteoclast differentiation dependent on the activation of free fatty acid receptor 2 (Montalvany-Antonucci et al. 2019).

Osteoclast generation and bone resorption are energy-consuming processes closely related to energy metabolism. Lemma demonstrated that the energy required for osteoclast differentiation is mainly from oxidative phosphorylation, while peripheral cellular activities associated with bone matrix degradation are powered by glycolysis (Lemma et al. 2016). In the study of Lucas, the protective effect of SCFAs on bone mass was associated with the inhibition of osteoclast differentiation and bone resorption (Lucas et al. 2018). Because propionate and butyrate induce a shift in the metabolic direction of osteoclasts, leading to enhanced glycolysis without significant changes in oxidative phosphorylation levels, resulting in downregulation of essential osteoclast genes, such as TNF receptor-associated factor 6 (TRAF6) and Nuclear factor of activated T-cells, cytoplasmic 1 (NFATc1), affecting RANKL-induced osteoclast differentiation, thereby reducing the number of osteoclasts and regulating bone homeostasis (Fig. 1, Lucas et al. 2018). Wauquier found that GPR40 receptor-deficient mice displayed osteoporosis-like symptoms, indicating that GPR40 receptors have a favorable impact on bone density and serving as a mediator of fatty acid-induced bone remodeling (Wauquier et al. 2013). An in vitro experimental study by Wallimann revealed that many genes involved in osteoclast differentiation after butyric acid treatment were found to be differently expressed in osteoclast precursor cells (Wallimann et al. 2021). Additionally, differentially expressed genes on osteoblast precursor cells can markedly lessen the production of osteoclasts and the activity of bone resorption (Wallimann et al. 2021). Based on the above findings, SCFAs are an effective regulator of osteoclast metabolism and bone homeostasis.

Conclusions

Due to the intensification of the livestock and poultry industries in recent years, the bone problem has become more prevalent. Nowadays, there is an urgent need for effective control and treatment of skeletal diseases, and it is especially important to develop reasonable preventive and therapeutic strategies. There has been evidence that SCFAs that regulate the gut microbiota indirectly can modulate bone metabolism and prevent bone loss directly, improving bone health. In order to support bone health, SCFAs modulate the immune system, calcium absorption, and bone cell regulation (Table 2). Therefore, a more effective approach for bone diseases or bone health may be found according to provided animals with appropriate SCFAs.

In animal production, SCFAs may be widely employed as feed additives, which would help animals build their bones and indirectly increase production efficiency. Targeting SCFA as an entrance point might lead to the development of novel treatment approaches for metabolic bone disorders since it plays a significant role in bone remodeling. Furthermore, increasing the SCFA content through the supplementation of probiotics, prebiotics, or natural active components would be a safe, effective and affordable therapy. Certainly, additional studies are required to identify the probiotics, prebiotic preparations or natural active components that enhance the SCFA concentration, and then to determine their optimal ratios and dosages for clinical application. In the future, it is anticipated that the potential contribution of SCFAs will be found with the advancement of biomedical technology.

Abbreviations

5-HT	5-Hydroxytryptamine
BMD	Bone mineral density
GIT	Gastrointestinal tract
GPRs	G protein-coupled receptor
HDAC	Histone deacetylase
IFN-γ	Interferon-γ
L. Rhamnosus	*Lactobacillus Rhamnosus*
L. Salivarius	*Lactobacillus Salivarius*
LPS	Lipopolysaccharide
NFATc1	Nuclear factor of activated T-cells, cytoplasmic 1
NF-κB	Nuclear factor kappa-B
NLRP3	NOD-like receptor protein 3
OPG	Osteoprotegerin
RANKL	Receptor activator for nuclear factor-κB ligand
SCFAs	Short-chain fatty acids
STAT1	Signal transducer and activator of transcription 1
Th cells	T helper cells
TNF-α	Tumor necrosis factor-α
TRAF6	TNF receptor-associated factor 6
Tregs	Regulatory T cells

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Authors' Contributions

S.C.H. contributed to the conceptualization, resources, funding acquisition, and revision and editing of the manuscript. Y.F.H. performed the literature review and drafted the manuscript. A.S. contributed to revision and editing of the manuscript. P.C. and K.L.L. collected the literature and reviewed the text. All authors contributed to the article and approved the submitted version. All authors have read and approved the final version of the manuscript.

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Declarations

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The author declares that they have no competing interests.

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