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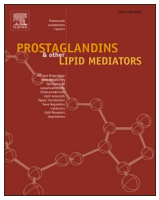

From tradition to science: Possible mechanisms of ghee in supporting bone and joint health

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ABSTRACT

Ghee, a traditional form of clarified butter, has been used for centuries in Ayurvedic medicine for its numerous health benefits. Recent scientific studies have begun to elucidate the molecular mechanisms by which ghee may support bone and joint health. This review explores the bioactive components of ghee, including short-chain fatty acids (SCFAs), medium-chain fatty acids (MCFAs), and fat-soluble vitamins (A, D, E, K2), and their potential therapeutic effects on bone density, joint lubrication, and inflammation. SCFAs in ghee can potentially improve joint lubrication and reduce inflammation. MCFAs and conjugated linoleic acid (CLA) exhibit anti-inflammatory properties, modulating cytokine production and oxidative stress pathways. Vitamins D and K2 in ghee can play potentially crucial roles in calcium metabolism and bone mineralization, while vitamin A supports immune regulation and cartilage health. This review integrates traditional knowledge with contemporary scientific research, highlighting the potential of ghee as a complementary therapy for conditions such as osteoporosis and arthritis. By understanding the molecular mechanisms involved, future studies can focus on this field to shed a light on different effects of ghee on bone and joint health.

1. Introduction

Ghee, a form of clarified butter that has been a main composition of Indian cuisine and Ayurvedic medicine for centuries, has received attention for its potential benefits [1]. Derived from cow's milk, this traditional food undergoes a steeping and skimming process to remove impurities and water content, resulting in a rich, golden liquid that is known for its unique flavor and smoke point (~250 °C) [2,3]. Beyond its culinary uses, ghee is valued in Ayurveda for its healing properties, including its benefits on the health of the nervous system, digestive system, skin, vision, respiratory system, cardiovascular system, and bones [1]. Although studies on the beneficial effect of ghee on bone and joint health are limited [4–6], with its active compounds, it can be hypothesized that ghee has a potentially beneficial effect on bone health. However, many studies are needed in this field.

One of the primary components that make ghee beneficial for bone health is its rich content of fat-soluble vitamins including vitamins A, D, E, and K2 [7]. Vitamin K2 is especially important for bone metabolism. It plays an important role in calcium regulation, ensuring that calcium is deposited in the bones and teeth instead of the arteries, thus increasing bone density and reducing the risk of osteoporosis and fractures [8]. The presence of vitamin D in ghee oil increases the absorption of calcium and increases its effectiveness in maintaining bone health [9]. Ghee oil also contains conjugated linoleic acid (CLA) [10], a type of fatty acid that has anti-inflammatory properties [11]. Chronic inflammation is a known cause of conditions such as arthritis [12]. In addition, the butyrate content of ghee oil is another aspect that is worth noting [13]. Butyrate is a short-chain fatty acid (SCFA) that serves as a key energy source for colon cells and has anti-inflammatory effects on the gut [14]. A healthy gut microbiome is essential for optimal absorption of nutrients, including those critical for bone health such as calcium and phosphorus [15]. Therefore, ghee's ability to support gut health indirectly contributes to stronger bones and healthier joints.

From an Ayurvedic point of view, ghee is a "rasayana" or a rejuvenating substance that promotes longevity and vitality. It is used to treat various ailments and is believed to increase the body's ability to heal and repair itself. Ayurvedic practitioners advocate the use of ghee oil for its benefits in lubricating joints, increasing flexibility, and reducing joint pain [1]. This traditional knowledge, along with modern scientific insights that require new studies, can lead to the inclusion of ghee oil as an

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adjunctive approach to bone and joint problems.

Despite these promising properties, it is important to approach ghee consumption in moderation. Ghee oil is rich in saturated fat and calories, and consuming too much of it can lead to adverse health effects such as weight gain and increased cholesterol levels [16]. Therefore, it is important to balance ghee consumption with other healthy eating practices.

The aim of this narrative mechanistic review is to investigate the mechanism of action of the potential active compounds of ghee oil in maintaining and enhancing skeletal health so that the current study can be a roadmap for future studies in this field. By bridging traditional knowledge and contemporary research, we seek to provide a comprehensive understanding of how this ancient superfood can contribute to modern health practices.

2. Types of ghee and nutritional composition

There are different types of ghee derived from different animal milks including cow, buffalo, goat, sheep, camel, mare, horse, *etc.* [1]. Cow ghee is the most common type of ghee used in various cuisines and traditional medicine systems like Ayurveda. Cow ghee is known for its golden yellow color, which comes from the presence of beta-carotene, a precursor of vitamin A [17]. Buffalo ghee is whiter and has a higher fat content compared to cow ghee [18]. A2 ghee is made from the milk of cows that produce A2 beta-casein protein, which is considered easier to digest and less likely to cause inflammation compared to A1 beta-casein protein [19]. Organic ghee is produced from the milk of cows that are raised on organic feed without the use of synthetic fertilizers, pesticides, or genetically modified organisms (GMOs) [18]. Desi ghee, known as traditional ghee, is made using traditional methods, often from cultured butter, which is churned from curdled milk. Desi ghee is praised for its authentic taste and higher nutritional value [20].

The nutritional composition of ghee can vary slightly depending on the type of milk used, the diet of the animals, and the method of preparation. Palmitic Acid (C16:0), stearic acid (C18:0), myristic acid (C14:0), and butyric Acid (C4:0) are predominant saturated fatty acids (SFAs) in cow, sheep, camel, and buffalo ghee. Instead of butyric Acid (C4:0), capric acid (C10:0) is more frequent in goat ghee. Oleic Acid (C18:1), linoleic Acid (C18:2), and linolenic Acid (C18:3) are almost predominant monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) in different types of ghee, respectively. Along with fatty acids, fat-soluble vitamins (A, D, E, and K2) and CLA can be mentioned as other main components of ghee [1].

3. Potential biological mechanisms of action of ghee on bone and joint health

Given mentioned bioactive compounds of ghee, some of the potential health benefits of ghee on bone and joint can be noted. These can be categorized as beneficial effects of ghee on bone density, joint health, and inflammation.

3.1. Bone density

Ghee contains significant amounts of fat soluble vitamins, which play a critical role in bone metabolism (Fig. 1). Vitamin K2 facilitates the carboxylation of bone proteins, including osteocalcin and matrix Gla-protein (MGP). Carboxylated osteocalcin binds to the bone mineral hydroxyapatite, strengthening the bone matrix. Vitamin K2 inhibits the activity of osteoclasts (bone-resorbing cells), reducing bone resorption and helping to maintain bone density [8]. This is particularly beneficial in conditions like osteoporosis where bone resorption outpaces bone formation. Vitamin K2 works synergistically with Vitamin D to enhance calcium absorption and utilization in bones [21]. Vitamin D increases the production of osteocalcin, which Vitamin K2 activates [21], thereby optimizing bone health. Therefore, the combination of vitamin D with vitamin K2 in ghee can potentially have a better effect than the administration of vitamin D or vitamin K2 alone on bone health.

Vitamin D is converted in the body to its active form, calcitriol. Calcitriol binds to vitamin D receptors in various tissues, including the

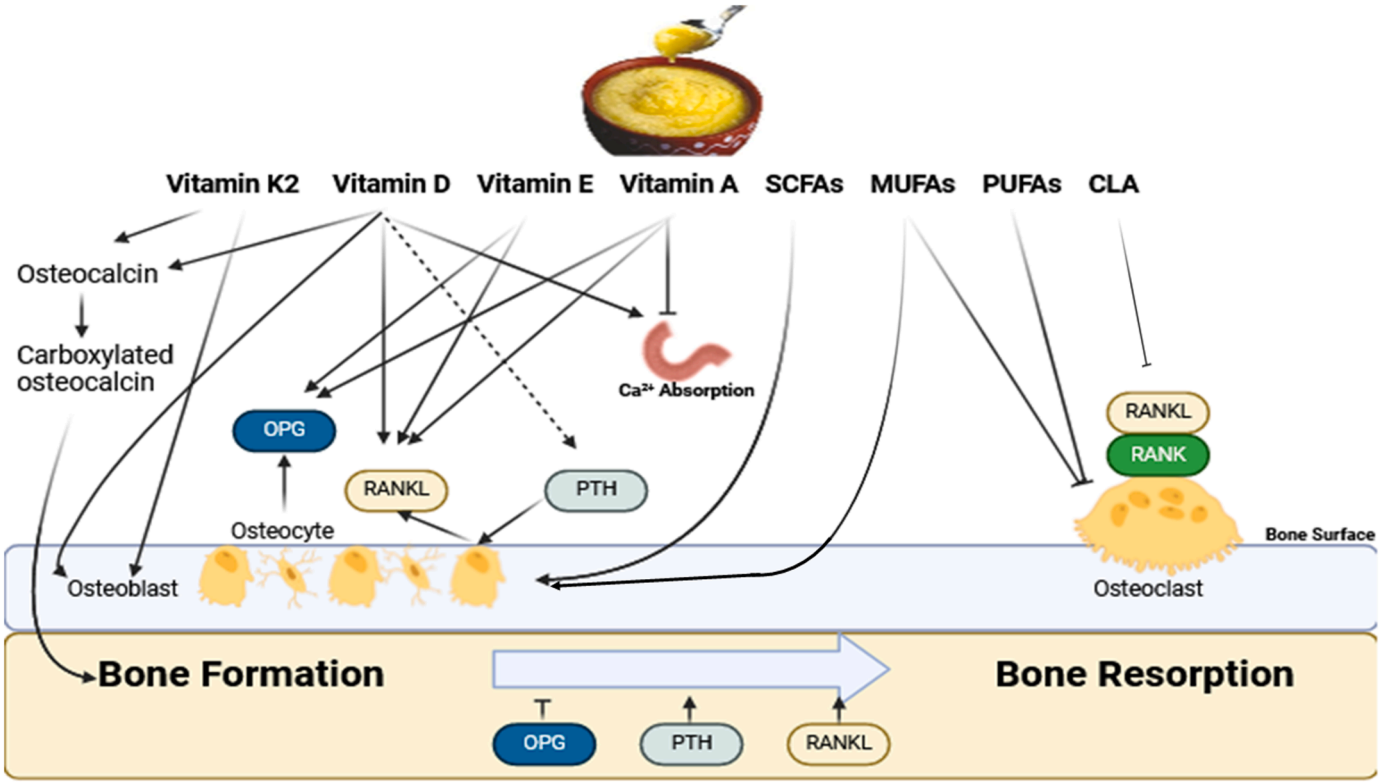

Fig. 1. The possible mechanism of ghee on bone metabolism.

intestines, bones, and kidneys, to regulate calcium and phosphate metabolism. This regulation is critical for maintaining bone homeostasis and promoting bone growth and remodeling. Calcitriol stimulates osteoblasts, the cells responsible for bone formation [9]. It regulates the expression of genes involved in bone matrix production and mineralization such as osteocalcin, osteopontin, calbindin, and receptor activator of nuclear factor kappa-B ligand (RANKL), thereby promoting bone growth and repair [22]. Vitamin D is crucial for the absorption of calcium in the intestines. It enhances the efficiency of calcium absorption, ensuring that enough calcium is available in the blood for bone formation and maintenance [23]. Moreover, vitamin D helps regulate the production of parathyroid Hormone (PTH), which in turn controls calcium levels in the blood. When calcium levels are low, PTH is released to increase calcium by resorbing it from bones. Adequate levels of vitamin D help keep PTH levels in check, preventing excessive bone resorption and maintaining bone density [24].

Vitamin E influences bone remodeling by regulating the expression of genes involved in osteoblast and osteoclast differentiation. The key signaling pathways of bone metabolism that be affected by vitamin E are RANK/RANKL/osteoprotegerin (OPG), Wnt/β-catenin, and oxidative stress signaling, thereby favoring bone formation over resorption [25].

Vitamin A plays a crucial role in bone health through its involvement in the regulation of osteoblast and osteoclast activity, which are essential for bone remodeling. At the molecular level, retinoic acid, the active metabolite of vitamin A, binds to nuclear receptors (RARs and RXRs) that regulate gene expression critical for bone formation and resorption. Retinoic acid modulates the expression of genes involved in the differentiation and function of osteoblasts, and osteoclasts. It enhances the synthesis of bone matrix proteins like collagen, while also promoting osteoclast differentiation and activity through the upregulation of RANKL and OPG, which are key regulators of osteoclastogenesis [26]. This dual action of vitamin A ensures balanced bone remodeling, which is essential for maintaining bone density and strength. However, vitamin A has been proposed to counteract vitamin D's role in enhancing calcium absorption and maintaining stable serum calcium levels. Consequently, elevated levels of vitamin A might impair vitamin D function [27]. Thus, vitamin A could have both beneficial and detrimental effects on bone health.

Ghee is a rich source of various fatty acids, including CLA, which are known for their potential benefits in promoting bone and joint health. The composition of these fatty acids can significantly influence the body's inflammatory responses, calcium metabolism, and overall skeletal health. SCFAs have been shown to activate the Wnt/β-catenin signaling pathway [28], which is crucial for osteoblast differentiation and bone formation [29]. By enhancing this pathway, SCFAs promote bone anabolic processes and improve bone density. SCFAs can also enhance the differentiation of mesenchymal stem cells into osteoblasts, the cells responsible for bone formation. This is mediated through various signaling pathways, including the activation of Runx2, a key transcription factor for osteogenesis [30].

MUFAs promote the differentiation and activity of osteoblasts. This process involves the upregulation of genes associated with osteogenesis, such as Runx2 and osterix, leading to increased bone matrix production and mineralization [31]. In addition, MUFAs reduce the formation and activity of osteoclasts. This is achieved by downregulating the expression of RANKL, a critical factor for osteoclast differentiation and activation [32].

Omega-3 PUFAs activate peroxisome proliferator-activated receptor gamma (PPARγ) [33], a nuclear receptor involved in the regulation of bone metabolism [34]. PPARγ activation promotes the expression of genes that enhance osteoblast differentiation and inhibit osteoclast activity, thereby improving bone density and strength [34]. Omega-3 PUFAs also improve calcium absorption in the intestines and enhance calcium deposition in bones. This effect is mediated through the upregulation of calcium transport proteins and the modulation of hormones involved in calcium homeostasis, such as calcitonin and parathyroid hormone [35].

CLA promotes the differentiation and activity of osteoblasts, the cells responsible for bone formation. This effect is mediated through the activation of PPARγ, which upregulates the expression of osteogenic genes such as Runx2 and osterix [36,37]. Enhanced osteoblast activity leads to increased bone formation and improved bone density. Moreover, CLA inhibits the formation and activity of osteoclasts. This is achieved by downregulating the expression of RANKL and upregulating the expression of OPG. By reducing osteoclast activity, CLA helps to maintain bone density and prevent bone loss [38].

3.2. Joint health

Vitamin K2 helps protect cartilage by promoting the synthesis of cartilage matrix proteins. This helps maintain the structural integrity of cartilage, which is essential for joint function and mobility [39].

Vitamin D receptors are present in cartilage tissue, and vitamin D helps maintain cartilage health by promoting the expression of cartilage-specific proteins, such as type II collagen and aggrecan. Type II collagen provides tensile strength to the cartilage matrix, while aggrecan, a proteoglycan, contributes to the compressive resilience by attracting water molecules, ensuring cartilage's ability to withstand mechanical stress [40]. Moreover, vitamin D signaling influences the expression of matrix metalloproteinases (MMPs), enzymes involved in the degradation of extracellular matrix components. By regulating MMP activity, vitamin D helps prevent excessive cartilage breakdown, thereby protecting against degenerative joint diseases such as osteoarthritis [41].

Vitamin E protects cartilage from oxidative damage and inflammation, both of which contribute to cartilage degradation in conditions like osteoarthritis. By preserving cartilage health, vitamin E helps maintain joint function and mobility. Moreover, vitamin E's antioxidant properties help to maintain the quality of synovial fluid, ensuring smooth joint movement and reducing the risk of joint deterioration [42].

Vitamin A contributes to the maintenance of cartilage, the flexible connective tissue in joints. It supports the health of chondrocytes, the cells responsible for cartilage production and maintenance, promoting joint health and function. Adequate vitamin A levels help preserve cartilage integrity and prevent joint deterioration [43].

SCFAs inhibit the activity of matrix metalloproteinases, enzymes that degrade cartilage extracellular matrix components such as collagen and proteoglycans. By suppressing MMP activity, SCFAs help preserve cartilage integrity and prevent joint degeneration [44].

By neutralizing reactive oxygen species (ROS), MUFAs protect chondrocytes (cartilage cells) from damage, thus preserving cartilage integrity and promoting joint health [45,46]. Omega-3 PUFAs stimulate the production of aggrecan and collagen, essential components of the cartilage extracellular matrix. This stimulation supports the maintenance and repair of cartilage, enhancing joint flexibility and resilience [47]. Additionally, omega-3 PUFAs improve the quality of synovial fluid, which reduces joint friction and wear [48].

CLA inhibits the activity of matrix metalloproteinases, enzymes that degrade the extracellular matrix components of cartilage such as collagen and proteoglycans. By suppressing MMP activity, CLA helps to preserve cartilage integrity and prevent joint degradation [49].

3.3. Inflammation

By modulating inflammatory cytokines and reducing oxidative stress, Vitamin K2 can alleviate symptoms of inflammatory joint diseases like arthritis. Vitamin K2 exerts its anti-inflammatory effects by downregulating pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-1beta (IL-1β), and IL-6, which are involved in the pathogenesis of arthritis. Vitamin K2 inhibits the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signaling pathway, which is a crucial regulator of these inflammatory cytokines, thereby reducing inflammation at the molecular level [50]. Moreover,

vitamin K2 enhances antioxidant defense mechanisms by upregulating the expression of antioxidant enzymes like superoxide dismutase (SOD) and glutathione peroxidase (GPx) [51]. These enzymes mitigate oxidative stress by neutralizing reactive oxygen species (ROS), which are known to exacerbate inflammation and joint damage in arthritis. By reducing oxidative stress, Vitamin K2 helps to protect joint tissues from oxidative damage and further inflammatory responses.

Vitamin D exhibits significant anti-inflammatory properties that can help reduce joint inflammation [52]. It achieves this by modulating the immune response, which involves influencing the activity of various immune cells. One of the primary ways vitamin D exerts its anti-inflammatory effects is by reducing the production of pro-inflammatory cytokines, such as TNF-α, IL-6, and IL-17 by inhibition of NF-κB [53], which play key roles in the pathogenesis of inflammatory joint diseases. Vitamin D also promotes the production of anti-inflammatory cytokines, such as IL-10, which helps to counterbalance the inflammatory response [54]. Furthermore, vitamin D can inhibit the differentiation and proliferation of T-helper 17 (Th17) cells, a subset of T cells that produce pro-inflammatory cytokines and are implicated in the pathogenesis of RA [55–57]. Additionally, vitamin D can enhance the regulatory T cell (Treg) population, which plays a critical role in maintaining immune tolerance and preventing autoimmune responses [58].

Vitamin E, particularly in the form of alpha-tocopherol, inhibits the activation of NF-κB. By suppressing NF-κB activation, vitamin E reduces the overall inflammatory response within bone and joint tissues [59]. Moreover, by scavenging ROS and enhancing the activity of endogenous antioxidant enzymes like SOD and GPx [60], vitamin E protects osteoblasts and osteoclasts from oxidative damage.

The anti-inflammatory action of vitamin A is mediated through its interaction with nuclear receptors such as RARs) and retinoid X receptors (RXRs). When vitamin A binds to these receptors, it influences the transcription of genes involved in the immune response, leading to a decreased production of inflammatory mediators and an enhanced production of anti-inflammatory molecules [61]. Furthermore, vitamin A promotes the differentiation and function of regulatory T cells (Tregs), which play a crucial role in maintaining immune tolerance and preventing excessive inflammatory responses [61,62]. In addition to its immunomodulatory effects, vitamin A's antioxidant properties help to protect joint tissues from oxidative damage, which can further exacerbate inflammation and joint destruction. By reducing oxidative stress, vitamin A helps preserve the integrity of joint cartilage and bone, contributing to overall joint health [63].

SCFAs, particularly butyrate, can inhibit the NF-κB pathway, a key regulator of inflammation. By suppressing NF-κB activation, SCFAs reduce the production of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6, which are involved in bone resorption and joint inflammation [64]. In addition, butyrate acts as a histone deacetylase (HDAC) inhibitor, leading to increased acetylation of histone proteins [65]. This epigenetic modification results in the transcriptional activation of anti-inflammatory and osteogenic genes, promoting bone formation and reducing inflammatory responses in joint tissues [66]. Moreover, SCFAs influence the gut microbiota composition, enhancing the growth of beneficial bacteria and reducing the abundance of pathogenic bacteria. This balance promotes a healthy gut barrier function and reduces systemic inflammation, which is beneficial for bone and joint health [67]. The gut microbiota modulation by SCFAs also affects the immune system, leading to a reduction in the activation of osteoclasts (bone--resorbing cells) and promoting osteoblast (bone-forming cells) activity [30].

Oleic acid, a primary MUFA in ghee, inhibits the NF-κB signaling pathway, which is crucial for the production of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6. This inhibition reduces inflammation in joint tissues, thereby protecting against joint degradation and bone resorption [68].

Omega-3 PUFAs, such as EPA and DHA, inhibit the synthesis of pro-inflammatory eicosanoids (e.g., prostaglandins and leukotrienes) derived from arachidonic acid [69]. This inhibition reduces joint inflammation and the progression of inflammatory joint diseases such as rheumatoid arthritis [70]. In addition, omega-3 PUFAs enhance the production of anti-inflammatory cytokines (e.g., IL-10) and promote the differentiation of T regulatory cells (Tregs), which help maintain immune tolerance and prevent autoimmunity [71]. This immunomodulatory effect is crucial for protecting joint tissues from immune-mediated damage.

CLA reduces the production of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6. This inhibition occurs through the modulation of the NF-κB signaling pathway, which is critical in the inflammatory response [72]. By decreasing the levels of these cytokines, CLA helps to reduce inflammation in joint tissues, protecting against conditions like arthritis. In addition, CLA can alter the metabolism of arachidonic acid, leading to a decrease in the synthesis of pro-inflammatory eicosanoids such as prostaglandins and leukotrienes [73]. This shift helps to reduce the inflammatory response in joints, promoting joint health and alleviating pain and stiffness associated with inflammatory joint diseases.

4. Evidence from studies

Limited number of studies have been performed about the effect of ghee on bone and joint health. However, by combining abovementioned compounds in ghee, it can be considered as a potentially beneficial compound to improve bone and joint health. Additional studies can shed a light on this field. A study examining the effects of Panchatikta Ghrita (PG) in rats demonstrated its anti-osteoporotic properties [6]. The term "Panchatikta" translates to "five bitter substances," indicating that the formulation contains five bitter herbs. These herbs are combined with ghee (clarified butter), which acts as a carrier to enhance the absorption and efficacy of the herbal ingredients. In rats induced with osteoporosis, PG administration resulted in improved serum calcium and inorganic phosphate levels, lower urinary calcium and phosphate excretion, a reduction in osteoclast numbers, and enhanced bone hardness compared to the untreated group [6]. Subsequent research by the same authors also indicated that PG effectively protects against postmenopausal osteoporosis [5], showing improvements in bone mineral density (BMD) scores, bone-specific biomarkers, and overall quality of life [4]. These findings suggest that PG holds potential as a preventive treatment for osteopenia.

5. Discussion

Ghee, a form of clarified butter originating from Indian cuisine, has been utilized in traditional medicine systems like Ayurveda for centuries. Its rich content of fat-soluble vitamins and unique fatty acid profile contribute to its potential health benefits [1]. Recently, scientific interest has grown in exploring the effects of ghee on bone and joint health. Traditional Ayurvedic texts have long touted the benefits of ghee for joint lubrication and overall bone health. Modern scientific studies are beginning to validate these claims, providing a bridge between traditional wisdom and contemporary evidence-based medicine. Ghee's unique composition, rich in beneficial fatty acids and fat-soluble vitamins, makes it a valuable addition to the diet for maintaining bone and joint health.

Ghee is composed of approximately 62 % saturated fats, 29 % monounsaturated fats (MUFAs), and 4 % polyunsaturated fats (PUFAs). It is rich in fat-soluble vitamins A, D, E, and K, and also contains conjugated linoleic acid (CLA) and butyrate, a short-chain fatty acid (SCFA) [1]. Each of these components plays a role in maintaining bone and joint health. However, ghee cannot be considered totally safe. Ghee is rich in saturated fats [1], which have been shown to increase low-density lipoprotein (LDL) cholesterol levels. Elevated LDL cholesterol contributes to the formation of atherosclerotic plaques within arterial walls [74]. Moreover, high-fat diets, including those rich in saturated fats like ghee,

can alter the composition of gut microbiota. These changes can lead to dysbiosis, characterized by a decrease in beneficial bacteria and an increase in pathogenic bacteria. Dysbiosis is linked to gastrointestinal discomfort, including bloating, indigestion, and diarrhea [75]. In addition, when ghee is heated to high temperatures, it can form trans fatty acids [76]. Trans fats are known to increase LDL cholesterol and decrease high-density lipoprotein (HDL) cholesterol, exacerbating the risk of cardiovascular diseases [77]. Moreover, ghee is highly caloric [1]. Excessive consumption can lead to an increase in overall caloric intake, resulting in positive energy balance and subsequent weight gain [1]. Over time, this can lead to obesity. In addition, ghee is low in omega-3 fatty acids, which are anti-inflammatory, and relatively higher in omega-6 fatty acids, which can be pro-inflammatory when not balanced with omega-3 s [1,78]. An imbalance favoring omega-6 can promote inflammation and is associated with an increased risk of chronic inflammatory diseases [78], including arthritis and cardiovascular diseases. Finally, it must be noted that although ghee is low in lactose and casein [79], individuals with severe dairy allergies or intolerances may still react adversely. However, some studies did not report any adverse effects by consuming ghee, even reported some beneficial effects on serum lipids compared with other types of oils [80,81]. Therefore, many studies are needed to clarify the different dimensions of the impact of ghee on bone and joint health.

6. Conclusion

The evidence suggests that ghee can potentially play a significant role in promoting bone and joint health through multiple mechanisms. Its rich content of vitamins D, K2, A, and E, along with beneficial fatty acids like CLA and butyrate, contributes to its anti-inflammatory, bone-strengthening, and joint-lubricating properties. While traditional knowledge has long recognized these benefits, modern scientific research has started to support the role of ghee as a functional food for bone and joint health. However, many studies are needed to clarify the different dimensions of the impact of ghee on bone and joint health. As mentioned, high consumption of ghee can have harmful effects due to the presence of saturated fatty acids.

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Conflict of interest

There are no conflicts of interest.

CRediT authorship contribution statement

Maryam Falahatzadeh: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Kaveh Bashti:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software. **Kianoosh Najafi:** Conceptualization, Funding acquisition, Investigation, Software, Visualization, Writing – original draft, Writing – review & editing.

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References

[1] D. Kataria, G. Singh, Health benefits of ghee: review of Ayurveda and modern science perspectives, J. Ayurveda Integr. Med. 15 (1) (2024) 100819.
[2] M.A. Afsaneh, M.A. Hosseinpour, A. Mina, Investigation of quality, advantages and disadvantages, processing and characteristics of ghee: a review paper, Indian J. Fundam. Appl. Life Sci. 6 (S2) (2016) 1–7.
[3] S. Deosarkar, C. Khedkar, S. Kalyankar, A. Sarode, Encyclopedia of food and health, butter, Manufacture (2016) 529–534.
[4] R.P. Munshi, D.A. Kumbhar, F.H. Panchal, P. Varthakavi, Assessing the effectiveness of panchatikta ghrita, a classical ayurvedic formulation as add-on therapy to vitamin D(3) and calcium supplements in patients with osteopenia: a randomized, open-labeled, comparative, controlled clinical study, J. Alter. Complement. Med. 25 (10) (2019) 1044–1053.
[5] R. Munshi, S. Joshi, F. Panchal, D. Kumbhar, P. Chaudhari, Does Panchatikta ghrita have anti-osteoporotic effect? Assessment in an experimental model in ovariectomized rats, J. Ayurveda Integr. Med. 12 (1) (2021) 35–42.
[6] R. Munshi, T. Patil, C. Garuda, D. Kothari, An experimental study to evaluate the antiosteoporotic effect of Panchatikta Ghrita in a steroid-induced osteoporosis rat model, Indian J. Pharm. 48 (3) (2016) 298–303.
[7] H.T. El-Gharrawy, K.M. Sadek, S.F. Mahmoud, A.M. Abd Elrehim, M. Shukry, H. I. Ghamry, S.F. Ibrahim, L. Fericean, M. Abdo, M.M. Zeweil, Natural ghee enhances the biochemical and immunohistochemical reproductive performance of female rabbits, Life (Basel) 13 (1) (2022).
[8] S. Akbari, A.A. Rasouli-Ghahroudi, Vitamin K and Bone metabolism: a review of the latest evidence in preclinical studies, Biomed. Res. Int. (2018) 4629383.
[9] E. Laird, M. Ward, E. McSorley, J.J. Strain, J. Wallace, Vitamin D and bone health: potential mechanisms, Nutrients 2 (7) (2010) 693–724.
[10] K. Chinnadurai, H.K. Kanwal, A.K. Tyagi, C. Stanton, P. Ross, High conjugated linoleic acid enriched ghee (clarified butter) increases the antioxidant and antiatherogenic potency in female Wistar rats, Lipids Health Dis. 12 (2013) 121.
[11] H.D. Putera, R.I. Doewes, M.N. Shalaby, A.A. Ramírez-Coronel, Z.S. Clayton, W. K. Abdelbasset, S.S. Murtazaev, A.T. Jalil, P. Rahimi, E. Nattagh-Eshtivani, M. Malekahmadi, N. Pahlavani, The effect of conjugated linoleic acids on inflammation, oxidative stress, body composition and physical performance: a comprehensive review of putative molecular mechanisms, Nutr. Metab. (Lond.) 20 (1) (2023) 35.
[12] D. Furman, J. Campisi, E. Verdin, P. Carrera-Bastos, S. Targ, C. Franceschi, L. Ferrucci, D.W. Gilroy, A. Fasano, G.W. Miller, A.H. Miller, A. Mantovani, C. M. Weyand, N. Barzilai, J.J. Goronzy, T.A. Rando, R.B. Effros, A. Lucia, N. Kleinstreuer, G.M. Slavich, Chronic inflammation in the etiology of disease across the life span, Nat. Med. 25 (12) (2019) 1822–1832.
[13] A.H.Md Asif, M.A.H. Sarker, G.K. Deb, M.R. Habib, S. Arefin, M.S. Bari, M.Z. Islam, M. Harun-Ur-Rashid, M.S.R. Siddiki, U.F. Shahjadee, S.A. Lisa, S. Ahmed, M. A. Islam, Fatty acid and amino acid profiles of cheese, butter, and ghee made from buffalo milk, J. Adv. Vet. Anim. Res. 9 (1) (2022) 144–154.
[14] V. Mansuy-Aubert, Y. Ravussin, Short chain fatty acids: the messengers from down below, Front. Neurosci. 17 (2023) 1197759.
[15] F. Inchingolo, A.M. Inchingolo, F. Piras, L. Ferrante, A. Mancini, A. Palermo, A. D. Inchingolo, G. Dipalma, The interaction between gut microbiome and bone health, Curr. Opin. Endocrinol. Diabetes Obes. 31 (3) (2024) 122–130.
[16] S.Mohammadi Hosseinabadi, J. Nasrollahzadeh, Effects of diets rich in ghee or olive oil on cardiometabolic risk factors in healthy adults: a two-period, crossover, randomised trial, Br. J. Nutr. 128 (9) (2022) 1720–1729.
[17] M.B. Wawre, D. Khobragade, D. Mundhada, An emerging approach for optimization of cow ghee as an ointment base in combination with selected conventional bases, Cureus 15 (3) (2023) e36556.
[18] S. Mor, V. Sharma, S. Arora, P.S. Minz, Physico-chemical and color parameters to distinguish cow ghee from buffalo ghee, J. Food Sci. Technol. 59 (8) (2022) 3231–3236.
[19] S. Jianqin, X. Leiming, X. Lu, G.W. Yelland, J. Ni, A.J. Clarke, Effects of milk containing only A2 beta casein versus milk containing both A1 and A2 beta casein proteins on gastrointestinal physiology, symptoms of discomfort, and cognitive behavior of people with self-reported intolerance to traditional cows' milk, Nutr. J. 15 (2016) 35.
[20] N. Ahmad, M. Saleem, Characterization of desi ghee obtained from different extraction methods using Raman spectroscopy, Spectrochim. Acta Part A: Mol. Biomol. Spectrosc. 223 (2019) 117311.
[21] A.J. van Ballegooijen, S. Pilz, A. Tomaschitz, M.R. Grübler, N. Verheyen, The synergistic interplay between vitamins D and K for bone and cardiovascular health: a narrative review, Int. J. Endocrinol. 2017 (2017) 7454376.
[22] I. Krela-Kaźmierczak, A. Szymczak, L. Łykowska-Szuber, P. Eder, K. Stawczyk-Eder, K. Klimczak, K. Linke, W. Horst-Sikorska, The importance of vitamin D in the pathology of bone metabolism in inflammatory bowel diseases, Arch. Med. Sci. 11 (5) (2015) 1028–1032.
[23] N. Khazai, S.E. Judd, V. Tangpricha, Calcium and vitamin D: skeletal and extraskeletal health, Curr. Rheuma Rep. 10 (2) (2008) 110–117.
[24] S.J. Khundmiri, R.D. Murray, E. Lederer, PTH and Vitamin D, Compr. Physiol. 6 (2) (2016) 561–601.
[25] S.K. Wong, N.V. Mohamad, N. Ibrahim, K.Y. Chin, A.N. Shuid, S. Ima-Nirwana, The molecular mechanism of vitamin E as a bone-protecting agent: a review on current evidence, Int. J. Mol. Sci. 20 (6) (2019).
[26] M.M.F. Yee, K.Y. Chin, S. Ima-Nirwana, S.K. Wong, Vitamin A and bone health: a review on current evidence, Molecules 26 (6) (2021).
[27] S. Johansson, H. Melhus, Vitamin A antagonizes calcium response to vitamin D in man, J. Bone Min. Res. 16 (10) (2001) 1899–1905.
[28] V. Rea, I. Bell, T. Ball, T. Van Raay, Gut-derived metabolites influence neurodevelopmental gene expression and Wnt signaling events in a germ-free zebrafish model, Microbiome 10 (1) (2022) 132.
[29] P. Duan, L.F. Bonewald, The role of the wnt/β-catenin signaling pathway in formation and maintenance of bone and teeth, Int. J. Biochem. Cell Biol. 77 (Pt A) () (2016) 23–29.

[30] Y. Kwon, C. Park, J. Lee, D.H. Park, S. Jeong, C.H. Yun, O.J. Park, S.H. Han, Regulation of bone cell differentiation and activation by microbe-associated molecular patterns, Int. J. Mol. Sci. 22 (11) (2021).
[31] J. Symmank, M. Chorus, S. Appel, J. Marciniak, I. Knaup, A. Bastian, C.L. Hennig, A. Döding, U. Schulze-Späte, C. Jacobs, M. Wolf, Distinguish fatty acids impact survival, differentiation and cellular function of periodontal ligament fibroblasts, Sci. Rep. 10 (1) (2020) 15706.
[32] B. van Heerden, A. Kasonga, M.C. Kruger, M. Coetzee, Palmitoleic acid inhibits RANKL-induced osteoclastogenesis and bone resorption by suppressing NF-κB and MAPK signalling pathways, Nutrients 9 (5) (2017).
[33] A.H. Faghfouri, Y. Khajebishak, L. Payahoo, E. Faghfuri, M. Alivand, PPAR-gamma agonists: potential modulators of autophagy in obesity, Eur. J. Pharm. 912 (2021) 174562.
[34] Y. Wan, PPARγ in bone homeostasis, Trends Endocrinol. Metab. 21 (12) (2010) 722–728.
[35] J.W. Nieves, Chapter 74 - Alternative Therapy through Nutrients and Nutraceuticals, in: R. Marcus, D. Feldman, D.W. Dempster, M. Luckey, J.A. Cauley (Eds.), Osteoporosis, Fourth Edition, Academic Press, San Diego, 2013, pp. 1739–1749.
[36] J. Kim, Y. Park, S.H. Lee, Y. Park, trans-10,cis-12 conjugated linoleic acid promotes bone formation by inhibiting adipogenesis by peroxisome proliferator activated receptor-γ-dependent mechanisms and by directly enhancing osteoblastogenesis from bone marrow mesenchymal stem cells, J. Nutr. Biochem. 24 (4) (2013) 672–679.
[37] Y. Han, C.Y. Kim, H. Cheong, K.Y. Lee, Osterix represses adipogenesis by negatively regulating PPARγ transcriptional activity, Sci. Rep. 6 (2016) 35655.
[38] M.M. Rahman, A. Bhattacharya, G. Fernandes, Conjugated linoleic acid inhibits osteoclast differentiation of RAW264.7 cells by modulating RANKL signaling, J. Lipid Res. 47 (8) (2006) 1739–1748.
[39] K.Y. Chin, The relationship between vitamin K and osteoarthritis: a review of current evidence, Nutrients 12 (5) (2020).
[40] R.J. Garfinkel, M.F. Dilisio, D.K. Agrawal, Vitamin D and its effects on articular cartilage and osteoarthritis, Orthop. J. Sports Med. 5 (6) (2017) 2325967117711376.
[41] S.K. Halder, K.G. Osteen, A. Al-Hendy, Vitamin D3 inhibits expression and activities of matrix metalloproteinase-2 and -9 in human uterine fibroid cells, Hum. Reprod. 28 (9) (2013) 2407–2416.
[42] K.Y. Chin, S. Ima-Nirwana, The role of vitamin E in preventing and treating osteoarthritis - a review of the current evidence, Front. Pharm. 9 (2018) 946.
[43] M. Enomoto, H. Pan, F. Suzuki, M. Takigawa, Physiological role of vitamin A in growth cartilage cells: low concentrations of retinoic acid strongly promote the proliferation of rabbit costal growth cartilage cells in culture, J. Biochem. 107 (5) (1990) 743–748.
[44] T. Kawamura, A. Andoh, A. Nishida, M. Shioya, Y. Yagi, T. Nishimura, T. Hashimoto, T. Tsujikawa, H. Yasui, Y. Fujiyama, Inhibitory effects of short-chain fatty acids on matrix metalloproteinase secretion from human colonic subepithelial myofibroblasts, Dig. Dis. Sci. 54 (2) (2009) 238–245.
[45] J.A. Bolduc, J.A. Collins, R.F. Loeser, Reactive oxygen species, aging and articular cartilage homeostasis, Free Radic. Biol. Med. 132 (2019) 73–82.
[46] L. Magtanong, P.-J. Ko, M. To, J.Y. Cao, G.C. Forcina, A. Tarangelo, C.C. Ward, K. Cho, G.J. Patti, D.K. Nomura, J.A. Olzmann, S.J. Dixon, Exogenous monounsaturated fatty acids promote a ferroptosis-resistant cell state, Cell Chem. Biol. 26 (3) (2019) 420–432.e9.
[47] K.D. Hankenson, B.A. Watkins, I.A. Schoenlein, K.G. Allen, J.J. Turek, Omega-3 fatty acids enhance ligament fibroblast collagen formation in association with changes in interleukin-6 production, Proc. Soc. Exp. Biol. Med. 223 (1) (2000) 88–95.
[48] M. Shawl, T. Geetha, D. Burnett, J.R. Babu, Omega-3 supplementation and its effects on osteoarthritis, Nutrients 16 (11) (2024) 1650.
[49] N.E. Hubbard, D. Lim, K.L. Erickson, Conjugated linoleic acid alters matrix metalloproteinases of metastatic mouse mammary tumor cells12, J. Nutr. 137 (6) (2007) 1423–1429.
[50] D. Mandatori, L. Pelusi, V. Schiavone, C. Pipino, N. Di Pietro, A. Pandolfi, The Dual Role of Vitamin K2 in "Bone-Vascular Crosstalk": Opposite Effects on Bone Loss and Vascular Calcification, Nutrients 13 (4) (2021).
[51] J. Nuszkiewicz, P. Sutkowy, M. Wróblewski, M. Pawłowska, R. Wesołowski, J. Wróblewska, A. Woźniak, Links between Vitamin K, Ferroptosis and SARS-CoV-2 Infection, Antioxidants 12 (3) (2023) 733.
[52] V. Musazadeh, Z. Kavyani, N. Mirhosseini, P. Dehghan, M. Vajdi, Effect of vitamin D supplementation on type 2 diabetes biomarkers: an umbrella of interventional meta-analyses, Diabetol. Metab. Syndr. 15 (1) (2023) 76.
[53] Y. Chen, J. Zhang, X. Ge, J. Du, D.K. Deb, Y.C. Li, Vitamin D receptor inhibits nuclear factor κB activation by interacting with IκB kinase β protein, J. Biol. Chem. 288 (27) (2013) 19450–19458.
[54] D.N. Roffe-Vazquez, A.S. Huerta-Delgado, E.C. Castillo, J.R. Villarreal-Calderón, A. M. Gonzalez-Gil, C. Enriquez, G. Garcia-Rivas, L. Elizondo-Montemayor, Correlation of Vitamin D with Inflammatory Cytokines, Atherosclerotic Parameters, and Lifestyle Factors in the Setting of Heart Failure: A 12-Month Follow-Up Study, Int J. Mol. Sci. 20 (22) (2019).
[55] S.H. Chang, Y. Chung, C. Dong, Vitamin D suppresses Th17 cytokine production by inducing C/EBP homologous protein (CHOP) expression, J. Biol. Chem. 285 (50) (2010) 38751–38755.
[56] M. Vitales-Noyola, B. Hernández-Castro, D. Alvarado-Hernández, L. Baranda, S. Bernal-Silva, C. Abud-Mendoza, P. Niño-Moreno, R. González-Amaro, Levels of Pathogenic Th17 and Th22 Cells in Patients with Rheumatoid Arthritis, J. Immunol. Res 2022 (2022) 5398743.
[57] V. Musazadeh, M. Zarezadeh, F. Ghalichi, F.H. Kalajahi, Z. Ghoreishi, Vitamin D supplementation positively affects anthropometric indices: Evidence obtained from an umbrella meta-analysis, Front. Nutr. 9 (2022) 980749.
[58] P. Srichomchey, S. Sukprasert, N. Khulasittijinda, N. Voravud, C. Sahakitrungruang, P. Lumjiaktase, Vitamin D(3) supplementation promotes regulatory T-cells to maintain immune homeostasis after surgery for early stages of colorectal cancer, Vivo 37 (1) (2023) 286–293.
[59] Y. Wang, N.Y. Park, Y. Jang, A. Ma, Q. Jiang, Vitamin E γ-tocotrienol inhibits cytokine-stimulated NF-κB activation by induction of anti-inflammatory A20 via stress adaptive response due to modulation of sphingolipids, J. Immunol. 195 (1) (2015) 126–133.
[60] E.D. Lewis, S.N. Meydani, D. Wu, Regulatory role of vitamin E in the immune system and inflammation, IUBMB Life 71 (4) (2019) 487–494.
[61] A.M. Pisoschi, A. Pop, F. Iordache, L. Stanca, O.I. Geicu, L. Bilteanu, A.I. Serban, Antioxidant, anti-inflammatory and immunomodulatory roles of vitamins in COVID-19 therapy, Eur. J. Med. Chem. 232 (2022) 114175.
[62] Z. Kavyani, V. Musazadeh, S. Golpour-Hamedani, A.H. Moridpour, M. Vajdi, G. Askari, The effect of Nigella sativa (black seed) on biomarkers of inflammation and oxidative stress: an updated systematic review and meta-analysis of randomized controlled trials, Inflammopharmacology 31 (3) (2023) 1149–1165.
[63] A. Mobasheri, H.K. Biesalski, M. Shakibaei, Y. Henrotin, Antioxidants and Osteoarthritis, in: I. Laher (Ed.), Systems Biology of Free Radicals and Antioxidants, Springer Berlin Heidelberg, Berlin, Heidelberg, 2014, pp. 2997–3026.
[64] D. Zhang, Y.-P. Jian, Y.-N. Zhang, Y. Li, L.-T. Gu, H.-H. Sun, M.-D. Liu, H.-L. Zhou, Y.-S. Wang, Z.-X. Xu, Short-chain fatty acids in diseases, Cell Commun. Signal. 21 (1) (2023) 212.
[65] S. Chriett, A. Dąbek, M. Wojtala, H. Vidal, A. Balcerczyk, L. Pirola, Prominent action of butyrate over β-hydroxybutyrate as histone deacetylase inhibitor, transcriptional modulator and anti-inflammatory molecule, Sci. Rep. 9 (1) (2019) 742.
[66] D. Bayarsaihan, Epigenetic mechanisms in inflammation, J. Dent. Res. 90 (1) (2011) 9–17.
[67] Y. Shin, S. Han, J. Kwon, S. Ju, T.G. Choi, I. Kang, S.S. Kim, Roles of short-chain fatty acids in inflammatory bowel disease, Nutrients 15 (20) (2023).
[68] C. Santa-María, S. López-Enríquez, S. Montserrat-de la Paz, I. Geniz, M.E. Reyes-Quiroz, M. Moreno, F. Palomares, F. Sobrino, G. Alba, Update on anti-inflammatory molecular mechanisms induced by oleic acid, Nutrients 15 (1) (2023).
[69] V. Musazadeh, A. Karimi, M. Malekahmadi, S.S. Ahrabi, P. Dehghan, Omega-3 polyunsaturated fatty acids in the treatment of non-alcoholic fatty liver disease: an umbrella systematic review and meta-analysis, Clin. Exp. Pharmacol. Physiol. 50 (5) (2023) 327–334.
[70] Z. Kavyani, V. Musazadeh, S. Fathi, A. Hossein Faghfouri, P. Dehghan, B. Sarmadi, Efficacy of the omega-3 fatty acids supplementation on inflammatory biomarkers: an umbrella meta-analysis, Int. Immunopharmacol. 111 (2022) 109104.
[71] S. Gutiérrez, S.L. Svahn, M.E. Johansson, Effects of omega-3 fatty acids on immune cells, Int. J. Mol. Sci. 20 (20) (2019).
[72] D. Dipasquale, L. Basiricò, P. Morera, R. Primi, A. Tröscher, U. Bernabucci, Anti-inflammatory effects of conjugated linoleic acid isomers and essential fatty acids in bovine mammary epithelial cells, Animal 12 (10) (2018) 2108–2114.
[73] J. Bulgarella, D. Patton, A. Bull, Modulation of prostaglandin H synthase activity by conjugated linoleic acid (CLA) and specific CLA isomers, Lipids 36 (2001) 407–412.
[74] P.W. Siri-Tarino, Q. Sun, F.B. Hu, R.M. Krauss, Saturated fatty acids and risk of coronary heart disease: modulation by replacement nutrients, Curr. Atheroscler. Rep. 12 (6) (2010) 384–390.
[75] A. Beam, E. Clinger, L. Hao, Effect of diet and dietary components on the composition of the gut microbiota, Nutrients 13 (8) (2021).
[76] S. Bhat, D. Maganja, L. Huang, J.H.Y. Wu, M. Marklund, Influence of heating during cooking on trans fatty acid content of edible oils: a systematic review and meta-analysis, Nutrients 14 (7) (2022).
[77] V. Dhaka, N. Gulia, K.S. Ahlawat, B.S. Khatkar, Trans fats-sources, health risks and alternative approach - a review, J. Food Sci. Technol. 48 (5) (2011) 534–541.
[78] J.J. DiNicolantonio, J. O'Keefe, The importance of maintaining a low omega-6/omega-3 ratio for reducing the risk of autoimmune diseases, asthma, and allergies, Mo Med. 118 (5) (2021) 453–459.
[79] P.A. Portnoi, A. MacDonald, The lactose and galactose content of milk fats and suitability for galactosaemia, Mol. Genet Metab. Rep. 5 (2015) 42–43.
[80] H. Sharma, X. Zhang, C. Dwivedi, The effect of ghee (clarified butter) on serum lipid levels and microsomal lipid peroxidation, Ayu 31 (2) (2010) 134–140.
[81] S. Manna, H.B. Sharma, S. Vyas, J. Kumar, Comparison of mustard oil and ghee consumption on the history of coronary heart disease in urban population of India, J. Clin. Diagn. Res. 10 (10) (2016). Oc01-oc05.