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Review Article

Lactoferrin in food and supplements and health benefits: state of the art and future directions

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Abstract: Lactoferrin, a protein well known for its iron-binding capacity, is abundant in colostrum but is less concentrated in mature milk. Lactoferrin is relatively resistant to the action of proteases in the gastrointestinal tract, especially in neonates, and a small proportion of lactoferrin can be absorbed in intact form. In randomized, controlled clinical trials, lactoferrin reduces invasive fungal infections in neonates and the prevalence of diarrhea in children. Lactoferrin receptors have been identified in different cell phenotypes, including notably intestinal and liver cells, osteoblasts and immune cells. Lactoferrin, which is generally recognized as safe (GRAS), has been shown in experimental studies to have antibacterial, antifungal, antiviral and antiparasitic effects. Beneficial effects of lactoferrin in intestinal and liver inflammation have been reported in experimental situations. In addition, oral supplementation with lactoferrin appears to be beneficial in experimental situations of compromised bone health. The present review recapitulates the effects of lactoferrin on target tissues in experimental and clinical situations. The present results highlight future research directions aimed at testing the potential utilization of this alimentary compound in different contexts.

Keywords: Lactoferrin, Dietary supplementation, Antimicrobial effects, Inflammation, Bone health

Introduction

Lactoferrin is a glycosylated globular protein that belongs to the transferrin family with iron-binding capacity [1-3]. Lactoferrin is present at relatively high concentration in maternal colostrum (~ 6 mg/mL) and consumed immediately by newborns after birth [4]. In mother's mature milk, the concentration of lactoferrin is generally lower, averaging between 2 and 3 mg of lactoferrin per mL of milk [5]. Infant milk formulas generally contain much lower amounts of lactoferrin (i.e. 0.1 mg/mL) when compared to mother's mature milk [6]. As other proteins found in mammalian milk, lactoferrin is sensitive to denaturation caused by infant formula processing, such as heat treatment [7-8], which has led to the development of minimally processed infant formula [9-10].

Lactoferrin is one of the major whey proteins and is found in bovine milk at a concentration of ~ 0.1 mg/

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mL [11]. Lactoferrin is also found in exocrine secretory products other than milk, but this latter aspect will not be discussed in this review. The biological effects of lactoferrin have been studied in humans following consumption of this protein present in diet and/or in supplements in different situations, and the reported consequences of such consumption have generally been considered beneficial. Lactoferrin originating from bovine milk is used as a food additive that is generally recognized as safe (GRAS) by the United States Food and Drug Administration [12]. The biological activities of lactoferrin rely on mechanisms of action that can be different depending on the targets considered.

The aim of this review is to provide an up-to-date overview of the published data obtained from clinical and experimental studies aiming at evaluating the effects of lactoferrin in different physiological and pathophysiological situations.

1. Lactoferrin can withstand digestion in the gastrointestinal tract and be partially absorbed in intact form

Lactoferrin is only partially digested by pepsin in the stomach. This partial digestion releases peptides with antibiotic capacity called collectively lactoferricin [13]. The biochemical properties of lactoferricin peptides enable them to interact with lipid compounds, as well as with negatively charged surfaces both of Gram-negative and Gram-positive bacteria, fungi, viruses and parasites, thus exerting antibacterial, antifungal, antiviral and antiparasitic effects [14-15]. Four decades ago, it was shown that lactoferrin is relatively resistant to the exocrine enzymatic protease activities of the pancreas [16]. A small proportion of lactoferrin is absorbed in its intact form by the intestine [17-18], and lactoferrin can be detected in the bloodstream after alimentary consumption [19]. Although the exact amplitude of lactoferrin degradation in the gastrointestinal tract remains unclear, it has been found that substantial amounts of lactoferrin can stay intact during gastric transit. Gastric pH and the presence of other proteins such as β-lactoglobulin modulate the extent of degradation in the gastrointestinal tract [20-22]. In fact, the timing of lactoferrin administration can modify its digestion, as the degradation of lactoferrin in gastric juice is different when it is administered prior or after a meal [23]. Furthermore, lactoferrin degradation in the gastrointestinal tract may be different according to the developmental stages. For instance, lactoferrin is not extensively digested in the gastrointestinal tract of newborns, resulting in the recovery of this protein in feces [24]. However, the amplitude of lactoferrin absorption remains debated [25], and it is assumed that approximately 10% of the lactoferrin present in the intestinal luminal fluid is absorbed [18]. However, this latter value, which is based on in vitro tests and in vivo assays in animal models, represents only a rough estimation of the actual intestinal absorption. Encapsulated lactoferrin appears to be more resistant to gastrointestinal proteases, thus representing an interesting and promising way of administration [26]. In healthy individuals, lactoferrin in the circulation is found in a concentration range between 2 and 7 µg/mL [27].

The presence of lactoferrin in the intestinal fluid and in the blood is consistent with the effects of this protein in several tissues, particularly in intestine, liver and bones. Lactoferrin performs various functions by binding to a wide range of receptors on target cells, resulting in a variety of biological responses. The target cells equipped with receptors to lactoferrin include intestinal epithelial cells [28-29], liver cells [30-31], osteoblasts [32], endothelial cells [33], fibroblasts [34], lymphocytes [35], macrophages [36] and platelets [37].

2. Lactoferrin displays numerous effects on the intestinal ecosystem

The small and large intestines are well known to be inhabited by a complex mixture of microbes, among which bacteria have been the subject of most studies. The concentration of bacteria in the large intestine is in the range of 10^9 to 10^{12} colony-forming unit (CFU) per g of content. The spectacular increase in the concentration of bacteria in the large intestine is mainly due to the fact that the intestinal content is transported much more slowly in the large intestine than in the small intestine, allowing intense metabolism of the available substrates supplied by the host [38]. This microbial population includes not only bacteria, but also archaea, viruses and fungi. Lastly, the protozoans in the intestine, although not classically included as part of the microbiota itself, represent a heterogeneous group of eukaryotic organisms, some of which are considered as parasites [39].

In numerous studies, it has been shown that lactoferrin, like lactoferricin (see above) can exert antibacterial, antiviral, antifungal and antiparasitic effects and contribute to the specific regulation of the immune response [40-42]. Lactoferrin can modulate cytokine production by intestinal immune cells [43-44].

The antimicrobial effect of lactoferrin is mainly attributed to its capacity to bind iron and thus allowing reduction of its availability to bacteria. However, other mechanisms of action have been shown to be presumably involved in the antimicrobial effect of lactoferrin, such as the presence of lactoferrin receptors in bacteria [45], the capacity of lactoferrin to bind to bacterial cell walls and thus to disrupt the integrity of bacteria and contributes to the bacterial death, as well as the direct interaction with Gram-negative bacteria leading to cell membrane damages [2, 46]. Interestingly, lactoferrin has

been shown for instance to affect Helicobacter pylori infection [47]. It is also interesting to note that bovine holo-lactoferrin can retard the growth of the pathogenic bacteria Clostridium difficile and reduce the production of toxins by these bacteria in a model of bacterial infection [48]. However, the global effects of lactoferrin on the intestinal bacteria composition and metabolic activity remain largely unknown [49]. As recently investigated by Li and collaborators, lactoferrin appears to act not only on enteropathogenic bacteria, but also as a prebiotic that induces changes in the intestinal ecosystem characteristics and in host intestinal physiology [50]. For instance, an experimental study with suckling piglets has shown that lactoferrin supplementation modifies the composition of the microbiota in the colon, an effect that was associated with an increased concentration of the bacterial metabolite butyrate in the colonic fluid and with an increased expression of genes involved in the maintenance of the colonic barrier function [51].

Regarding the antiviral effect of lactoferrin, this protein has been demonstrated to inhibit the growth of both DNA and RNA viruses, in particular human immunodeficiency virus (HIV), human cytomegalovirus (HCMV), rotavirus, respiratory syncytial virus and herpesviruses [52-53]. Dietary supplementation with lactoferrin may represent an adjunctive treatment to medication for the treatment of viral infection [54]. The mechanisms by which lactoferrin exerts its antiviral effect remains unclear, but interestingly, this protein has been shown to bind to receptors such as ACE2, which are used by SARS-CoV-2, thus reducing the attachment of the viral pathogen to the host cells [55-56]. The fungicidal activity of lactoferrin has been shown against various Candida species, including Candida albicans [57-58]. Furthermore, the antiparasitic effect of lactoferrin has been demonstrated in vitro against the malaria parasite Plasmodium falciparum [59] and in vivo and in vitro against the protozoan parasite Toxoplasma gondii [60]. Lastly, microbicidal effects of lactoferrin-derived peptides have been demonstrated against the parasite Entamoeba histolytica [61].

Lactoferrin is bioactive at different stages of development. In newborns, lactoferrin modulates the intestinal permeability and exerts a trophic effect on the mucosa [62]. These effects have been similarly observed in an in vitro study and in an in vivo animal study [63-64]. Furthermore, lactoferrin efficiently increases the proliferation and differentiation of intestinal cells in an in vivo rodent model and in an in vitro study with intestinal epithelial cells [65]. Moreover, it was shown that the ingestion of lactoferrin by the mother during gestation and lactation promotes the early development of the pups in a rodent model [66]. This latter effect on pups coincided with an increased differentiation of small intestine epithelial cell and an increased colon barrier function. The increased epithelial cell differentiation and the increased expression of genes coding for the tight-junction proteins were associated with higher plasma amino acid concentrations. In two randomized controlled trials, lactoferrin supplementation was found to exert beneficial effects on children by reducing the prevalence and severity of diarrhea [67] and to prevent invasive infection in neonates [68]. In the first trial which was randomized, controlled and double-blinded, 555 children enrolled at 12-18 months were given bovine lactoferrin (0.5 g twice a day) or placebo for six months (half children in the lactoferrin group and half children in the placebo group). Lactoferrin supplementation was associated with a reduction in the longitudinal prevalence of diarrhea ($p = 0.0046$), a reduction in the median duration of diarrhea episodes ($p = 0.0046$), a reduction in liquid stool load ($p < 0.001$), and a reduction in episodes of dehydration ($p = 0.0045$) [67]. In the second randomized controlled trial involving 472 neonates, it was found that 6 weeks administration of bovine lactoferrin (0.1 g/day) to very low birth weight infants reduced invasive fungal infections compared to the placebo group ($p = 0.002$) [68].

3. Lactoferrin exerts anti-inflammatory effects in situation of intestinal inflammation

Oral administration of lactoferrin in animal models of colitis alleviates the symptoms of intestinal inflammation by modulating the immune system and reducing the production of pro-inflammatory cytokines in colonic tissue [69-71]. In addition, lactoferrin and its derived peptides reduce the signs of colitis in mice when administered orally [72]. Indeed, in this latter experiment, lactoferrin was able to reduce the presence of occult blood in the feces and the number of tumor necrosis factor alpha (TNF-α)-producing cells in the distal colon. Furthermore, in the model of colitis induced by dextran sulfate sodium (DSS), bovine lactoferrin was found to reduce inflammation and impairment of colonic epithelial barrier function [73]. Interestingly, oral administration of a Lactococcus lactis strain that secretes lactoferrin-derived peptides was found to alleviate the development of acute colitis in mice [74].

Lactoferrin supplementation was also tested in a model of in vivo endotoxemia induced by administration of lipopolysaccharide (LPS) which is characterized by a systemic inflammatory response [75]. LPSs are bacterial surface glycolipids produced by Gram-negative bacteria which can be transferred from the luminal intestinal fluid into the bloodstream. At the intestinal level, systemic administration of LPS increases intestinal permeability, epithelial cell apoptosis and shedding, while causing villus shortening and diarrhea [76-79]. These effects of LPS are associated with reduced intestinal mucosa oxygen consumption and intestinal absorption of amino acids [80-81]. Lactoferrin alleviates LPS-induced inflammation in different in vivo experimental models [82-85], and lactoferrin deficiency further aggravates LPS-induced

inflammation [86]. From a mechanistic point of view, lactoferrin and its derived peptides inhibit the secretion of pro-inflammatory cytokines through different actions, including sequestration of LPS [87-88] and inhibition of the binding of nuclear transcription factor kappa B (NF-κB) to the TNF-α promoter following cellular uptake of lactoferrin [89]. Lactoferrin can apparently bind directly to intact LPS [90] and to bacterial LPS on the bacterial surface [91]. Lactoferrin supplementation, when performed before LPS administration, has been recently shown to reduce the circulating concentration of the inflammatory cytokine TNF-α, improve intestinal permeability, and maintain the morphology of the intestinal mucosa [90].

Iron deficiency anemia is often associated with inflammatory bowel diseases [92]. In a randomized clinical trial involving 80 children with anemia associated inflammatory bowel disease, it was found that lactoferrin (0.1 g/day) was more effective than ferrous sulfate (6mg/kg/day) when both supplements were administrated for 3 months, on blood hemoglobin ($p < 0.001$), serum iron ($p < 0.001$) and serum ferritin concentration ($p = 0.006$) [93].

Incidentally, the amount of lactoferrin in the feces is considered representative of activated neutrophil infiltration in the case of intestinal inflammation and thus represents a fecal marker for such inflammation [94]. It has been shown that the amounts of lactoferrin released by neutrophils correlate with the severity of inflammation in the gastrointestinal tract [95]. The respective prognostic value of lactoferrin and another fecal indicator, namely calprotectin, has been discussed [96]. Although the lactoferrin release from neutrophils is endogenous but not from dietary origin in that case, the presence of lactoferrin in fecal material suggests that this protein is not extensively degraded by the intestinal microbiota.

4. Lactoferrin exerts anti-inflammatory effects in situation of liver inflammation

The fact that lactoferrin can bind to hepatocytes and in particular to plasma membranes, and that this protein can be taken up by liver cells [97-99] has motivated studies on the effects of lactoferrin on the liver in different experimental situations in recent years. In mice receiving the contaminating mycotoxin deoxynivalenol, lactoferrin has anti-inflammatory and antioxidative effects on the liver by regulating nuclear factor-E2-related factor 2/mitogen-activated protein kinase (Nrf2/MAPK) signaling pathways [100]. In a rodent model of non-alcoholic steatohepatitis, lactoferrin prevents hepatic fibrosis via the inhibition of NF-κB signaling [101]. In another model of liver damage provoked by acute alcohol consumption, lactoferrin was shown to improve the redox-stress response and thus to reduce liver damage [102]. The beneficial effects of lactoferrin on liver injury induced by ethanol consumption have been shown to be associated with nuclear translocation of Nrf2 in a rat model [103].

Beneficial effects of lactoferrin were also recorded in models of liver injury induced by adverse dietary conditions. Xiong and collaborators have shown that lactoferrin attenuates high-fat diet-induced hepatic steatosis by limiting hepatic lipogenesis in association with a reduction in inflammatory state [104]. Finally, Liu and collaborators have obtained encouraging results regarding the use of lactoferrin-producing bacteria in case of liver injury [105]. Indeed, lactoferrin produced by lactic acid bacteria was found to be beneficial in mice with non-alcoholic fatty liver disease, since it diminished the hepatic steatosis in such experimental situation.

5. Lactoferrin exerts beneficial effects on bones

Using ovariectomized mice as a model for the postmenopause, Blais and collaborators found that this protein was recovered in the peripheral blood after ingesting lactoferrin in the diet, thus being partially absorbed. Such supplementation allows to ameliorate bone mineral density and to increase the strength on the femurs [106]. In this latter study, lactoferrin stimulated the growth and differentiation of osteoblastic cells while reducing the growth of preosteoclastic cells. These beneficial effects of lactoferrin on bone physiology were found to be associated with modulation of lymphocyte activation and cytokine release in the bone microenvironment. This suggests that lactoferrin exerts its beneficial effects on postmenopausal bone loss partly by modulating immune functions [107]. Lactoferrin increases the proliferation of osteoblast-like cells as shown by thymidine incorporation into these cells, as well as their differentiation. Furthermore, lactoferrin reduces the apoptosis of osteoblast-like cell and at the same time reduces osteoclastogenesis [108]. In vitro, lactoferrin enhanced osteogenesis of adipose-derived stem cells [109]. Lactoferrin-derived peptides treated with pepsin have been shown to stimulate osteoblast proliferation [110], indicating that lactoferrin-derived peptides may be involved in the overall beneficial effects of lactoferrin on bone physiology. Finally, in the rat model, bone mineral density was higher in pups from mothers who received the lactoferrin supplements during gestation and lactation periods than in their counterparts in control group [66], which suggests that the dietary consumption of lactoferrin by mothers has beneficial effects on bone physiology in offspring.

In a recent study, using a new experimental model of ovariectomized mice with moderate dietary protein restriction, Blais and collaborators have shown that oral supplementation with lactoferrin significantly improves bone properties in this model characterized by severely

impaired bone quality [111], while such supplementation was found effective in maintaining bone mass and microarchitecture in ovariectomized rats [112]. The in vitro and in vivo experimental studies on the effects of lactoferrin on bone cells and bone properties suggest potential therapeutic effects of this protein and its derived peptides for some bone-related diseases including osteoporosis [113]. Lactoferrin signaling in osteoblasts includes low density lipoprotein receptor-related protein-1 (LRP-1), transforming growth factor-β (TGF-β) and insulin-like growth factor-1 (IGF-1), which activate downstream pathways such as extracellular signal-regulated kinase (ERK), phosphoinositide 3-kinase/protein kinase B (PI3K/Akt) and NF-κB that are involved in osteoblast proliferation, differentiation and mineralization [113].

6. Conclusion and future directions

The available experimental arguments indicate that lactoferrin is bioactive in different situations on different prokaryotic and eukaryotic cells, including bacteria, fungi, parasites, intestinal and hepatic cells, and bone cells; and the effects obtained are generally considered as beneficial. Figure 1 summarizes the main beneficial effects of lactoferrin.

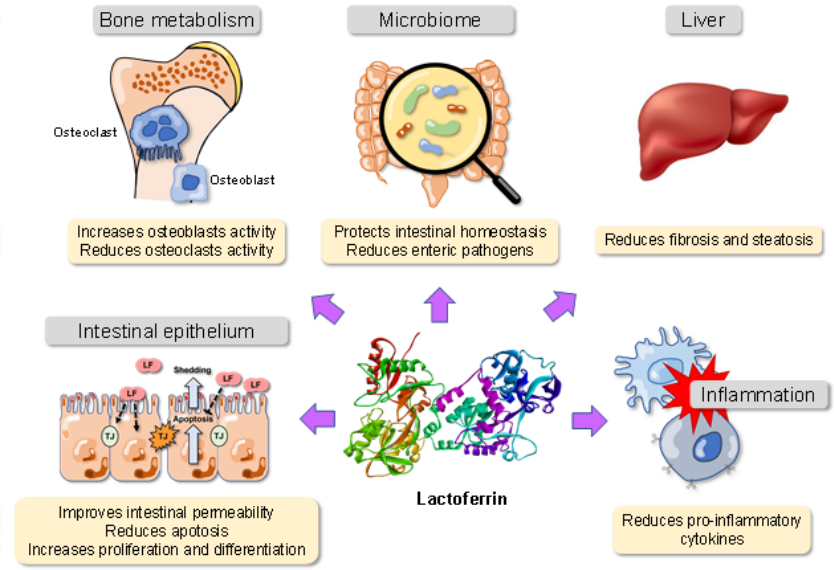

Figure 1. Schematic representation of the main beneficial effects of lactoferrin

Table 1. Proposed future directions for research on lactoferrin

Questions	Strategy
How is lactoferrin distributed between the intestinal luminal fluid and bloodstream according to the amounts in supplements?	Measurement of lactoferrin in feces and blood at different time after supplementation
What is the resistance of lactoferrin to bacterial proteases?	Measurement of lactoferrin degradation in the presence of intestinal microbiota
Can lactoferrin modify the intestinal microbiota?	Measurement of the fecal microbiota composition after lactoferrin supplementation
Can lactoferrin be beneficial in situation of liver injury and intestinal inflammation?	Measurement of the effects of lactoferrin in clinical trials
Is lactoferrin efficient for the prevention and/or treatment of osteoporosis?	Effects of lactoferrin supplementation in volunteers at risk for osteoporosis
Are lactoferrin-derived peptides efficient in inflammatory situations?	Identification, characterization, and testing of peptides derived from lactoferrin in inflammatory diseases

However, it is obvious that further research is needed to convert the promising results obtained from experimental works with animal and cell models into applications in real life. The proposed research directions are presented in Table 1.

First, as indicated in Table 1, we need to know more about the distribution of ingested lactoferrin between the intestinal luminal fluid and the bloodstream according to the dose of lactoferrin used. Such results will help us to understand the origin of lactoferrin, either from the intestinal contents or from the bloodstream, for the observed effects. In particular, it would be much helpful to determine the concentration of lactoferrin in the small and large intestine after dietary supplementation. If as some experimental studies suggest, lactoferrin is partially resistant to proteases in the gastrointestinal tract, it can be assumed that part of the lactoferrin is transferred from the small to the large intestine through the ileocecal junction. The resistance of lactoferrin to proteases of the intestinal microbiota also needs to be tested. In pre-clinical experimental studies with rodents and pigs, different doses of orally administrated bovine lactoferrin could be tested for the concentration of lactoferrin and its degradation products in blood and luminal fluid obtained from the different parts of the small and large intestine at different time points after oral ingestion. Indeed, rodents and pigs are a useful model for studies on the effects of dietary supplementation on metabolic and physiological parameters [114]. Randomized, controlled clinical studies with increasing doses of oral lactoferrin and assay of lactoferrin in feces and blood in healthy subjects will be obviously also useful to answer this question.

We also need to better know the effects of lactoferrin in oral supplements on microbiota composition and metabolic activity. Although the available results clearly indicate an antimicrobial effect of lactoferrin, we do not know the effects of increasing amounts of oral lactoferrin on fecal microbiota composition and metabolic activity. The efficacy of lactoferrin in the cases of infection with pathogenic bacteria, fungi, viruses and parasites could usefully be tested in animal models. Animal models of infection with these microbes could be tested for the efficacy of different doses of orally delivered lactoferrin on microbial infection kinetics. The encouraging results obtained after oral supplementation with lactoferrin in experimental intestinal inflammation and liver steatosis motivate the test of the utilization of lactoferrin as an additional treatment in clinical trials with volunteers prone to chronic inflammation of the intestinal mucosa and liver injury. Although it is obvious that lactoferrin per se cannot substitute for pharmacological treatment in patients with steatosis or chronic inflammatory bowel diseases, lactoferrin supplementation may prove effective as an adjunctive therapy in future randomized, controlled clinical trials.

The effects of oral supplementation with lactoferrin on bone properties in experimental studies urge for testing this protein in clinical situations of impaired (and risk of impaired) bone health. For instance, it would be of interest to test the effect of different doses of orally delivered bovine lactoferrin in volunteers prone to osteoporosis. The potential synergistic effects of lactoferrin with other dietary components such as total dietary proteins, calcium and vitamin D also remains to be tested in experimental and clinical trials. Interestingly, in the mouse model, lactoferrin supplementation enhances the expression of the vitamin D receptor in bone, an effect associated with increased bone mineral density [115]. These results raise the view that lactoferrin and vitamin D may act in synergy to promote bone health.

Secondly, progress in the identification of lactoferrin-derived peptides and their biochemical (heat resistance, resistance to bacterial proteases) and physiological properties (absorption through the intestinal epithelium, dose-response curves on pathophysiological parameters) would help to select the most efficient peptides in given situations of inflammatory states, allowing then the tests of the efficacy of these compounds in experimental and clinical studies.

Finally, it should be noted that the absorption and bioavailability of lactoferrin may be different in different subpopulations such as the newborn, infant and the elderly, but little information is available on that topic [116]. Furthermore, still limited evidence is available from clinical trials on the biological effects of lactoferrin since most of studies published are related to experimental preclinical studies. Lastly, possible side effects of lactoferrin supplementation need to be considered and investigated to fully evaluate the potential of lactoferrin in clinical applications in the different situations mentioned above.

Conflict of interest

The authors declare there is no conflict of interest.

Authors' contribution

Both authors participated equally in the writing of this review.

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