

## Lactoferrin – a promising bone-growth promoting milk-derived glycoprotein

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### Summary

Lactoferrin, an iron-chelating protein present in the granules of polymorphonuclear leukocytes and in the milk whey is having powerful osteogenic activity, as demonstrated by experiments performed *in vitro* on osteoblast cell cultures and *in vivo* on mice. It seems to be a promising factor to be applied in osteoporosis treatment.

**Key words:** lactoferrin, osteoblasts, osteogenesis, bone resorption, neutrophils

### Laktoferyna – glikoproteina o działaniu proosteogennym

#### Streszczenie

Laktoferyna, białko chelatujące żelazo, obecne w ziarnistościach granulocytów obojętnochłonnych, a także we frakcji białek serwatkowej mleka, a więc produkt gruczołów mleknych, wykazuje wybitne działanie osteogenne, zarówno wobec komórek osteoblastycznych w hodowli *in vitro*, jak również w sytuacji doświadczeń *in vivo*. Z przeglądu dostępnych informacji można laktoferynę uznać jako obiecujący środek dla pobudzania kościotworzenia *in vivo* i jako preparat w leczeniu i zapobieganiu osteoporozy, bez względu na jej przyczynę.

**Słowa kluczowe:** laktoferyna, osteoblasty, osteogeneza, resorpcja kości, neutrofile

The pharmacological treatments of choice for osteoporosis patients, regardless of the causal factor is the use of antiresorptive agents – bisphosphonates, and estrogen. This medication is not fully effective however, and there is a need for bone-anabolic agents and for osteogenic agents.

Lactoferrin, a 80 kD iron-binding glycoprotein, is structurally similar to transferrin, the plasma iron transport protein, but lactoferrin has a much higher (250 fold) affinity for iron. It is made by salivary glands, lacrimal gland, mucosal epithelium and by neutrophils, being a major constituent of secondary granules of polymorphonuclear cells. Lactoferrin is released by these cells in response to inflammatory stimuli

This iron-chelator has antimicrobial activity. Bacterial growth is inhibited by its ability to sequester iron and also permeabilize bacterial cell walls by binding to lipopolysaccharides through its N-terminus, [1], and affects immunity [2, 3] and many other processes [4]. This glycoprotein is also synthesized by the mammary gland and therefore is present in the milk as a fraction of whey protein. Thus, the whey is a rich and easily available source of this pleiotropic glycoprotein, and now is commercially available.

Lactoferrin was recently discovered to be a potent bone promoting agent, exhibiting this activity both, *in vivo* and *in vitro*. Lactoferrin's effects on bone have been extensively studied by group from Auckland University [5-8]. They established that bovine and human lactoferrin, purified from milk, stimulated proliferation of primary rat and human osteoblasts, as well as of bone marrow stromal cells and human established SaOS-2 osteoblast-like cells derived from osteosarcoma in a dose-dependent manner. This stimulatory effect, evaluated by 3H-thymidine incorpora-

tion, was observed at the physiological range of lactoferrin concentration. This effect was not species specific and cross-reacted between species.

Lactoferrin has also stimulatory effect on chondrocytes in primary cultures [6]. An addition of lactoferrin into medium that has induced osteoblastic differentiation increased the number of mineralizing bone nodules in primary cultures of rat osteoblast [6] and human osteoblast-like cells [9]. Lactoferrin incorporated into collagen membrane was a good adhesive substrate for growth and osteogenic differentiation of human MG63 osteoblastic cell line, as indicated by an increase of alkaline phosphatase activity and the synthesis of osteocalcin, both considered as osteogenesis markers. These results suggest that the collagen membranes could be useful as a drug delivery carrier for lactoferrin in bone tissue engineering [10].

Another way to expand the population of osteoblast in the *in vitro* cultures is the inhibition of their apoptosis by lactoferrin.

Lactoferrin administered at low concentration (10 ug/ml) to mouse bone marrow cultures significantly decreased a development of multinucleated, TRAP-positive cells (osteoclasts), however it did not affect bone-resorbing activity of isolated osteoclasts cultured *in vitro* even at higher doses of lactoferrin. Thus the presence of lactoferrin does not affect the activity of mature osteoclasts, but profoundly inhibit the generation of osteoclasts from mononuclear precursor cells.

Lactoferrin increases bone formation *in vivo*. Local administration of this protein on mouse skull bones resulted in significant apposition of new bone over the lactoferrin-exposed calvaria within 2 weeks.

On the basis of in vitro results of the application of lactoferrin on osteoblast cultures, it seems reasonable that a potent effect of this protein, administered locally, on bone growth is the manifestation of an anabolic effect of lactoferrin on osteoblasts and the prevention of osteoclastogenesis and inhibition of osteoblast apoptosis [5, 6].

Lactoferrin, as other growth factors, activates osteoblasts following binding to its receptor. Lactoferrin receptors belong to the endocytic LPR family (lipoprotein receptor-related protein). Human osteoblasts express LPR receptors and lactoferrin is its specific inhibitor [10]. Through this receptor also lipoproteins and vitamin K are delivered to osteoblast. Lactoferrin/receptor complex is internalized by osteoblastic cells within 30 minutes [5].

Lactoferrin induces phosphorylation (activation) of MAPk (mitogen activated kinase) p42/44 (Erk), and consequently stimulates osteoblasts proliferation, while receptor associated protein –RAP–inhibits lactoferrin-induced proliferation. LPR receptors, activated by lactoferrin binding, function as a mitogenic signaling receptor.

LPR receptors are associated with a protein – receptor associated protein (RAP), which specifically inhibits lactoferrin binding to the LPR receptors and inhibits internalization of the ligand/receptor complex.

Lactoferrin maintains osteogenic activity in various forms (deglycosylated, apo- and holoforms) and in various fragments, suggesting that lactoferrin signals through more than one receptor to exert its anabolic effect, and that it signals through diverse pathways [7, 8].

Lactoferrin seems to be a very promising agent for local stimulation of osteogenesis. Its systemic influence on the skeletal system and its possible application in osteoporosis treatment will be the subject of clinical testing. Iron loaded persons are also developing osteoporosis, as this metal ions suppresses bone remodeling by decreasing osteoblast formation and osteoid synthesis. Thus, iron chelator – lactoferrin – may be useful in prevention of osteoporosis [12].

Recently it was reported that supplementation of postmenopausal women with modified lactoferrin has beneficial effect on skeletal system: reduction of bone resorption, increase in osteoblastic bone formation and on restoration the balance of bone turnover within a short period [13].

Osteoblast stimulating activity of lactoferrin can at least partly explain the proliferation of bone at sites of inflammatory reaction with polymorphonuclear cells infiltration. Degranulation of neutrophils is an important element of reparative changes during bone fracture healing in rats [14]. In the phenomenon of ectopic endochondral osteogenesis by demineralized rat bone matrix implanted into muscles, the degranulation of polymorphonuclear leukocytes invading the implant bed is critical for bone induction to occur. Loss of the ability of demineralized bone matrix to induce degranulation of neutrophilic granulocytes correlated positively with loss of the ability to induce bone histogenesis [15].

Human lactoferrin hLF 1-13 when applied locally as an antimicrobial peptide for fighting osteomyelitis in the rat

femur showed bone ingrowth into Staphylococcus evoked lesions, suggesting a stimulatory effect of lactoferrin on osteoblasts and a toxic effect on Staphylococcus aureus cells [16].

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