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# Development of an enteric-coated lactoferrin tablet and its application

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## Discovery of novel physiological roles of lactoferrin

The ubiquitous presence of lactoferrin(LF) receptor in human as reported by the research group of Prof. Bo Lonnerdal, Univ. California (Suzuki *et al.* 2001), encouraged us to search for the unknown physiological roles of LF. Under the collaboration with Prof. Etsumori Harada, Tottori Univ., and his research group, we have found such two novel biological activities of LF as the control of the lipid metabolism and the effect on the central nervous system.

Relating to the lipid metabolism, LF could, in animal experiments, reduce triglycerides and total cholesterol both in blood and liver (Takeuchi *et al.* 2003). LF increased plasma HDL-C and lowered LDL-C.

In the central nervous system, LF showed antinociceptive activity mediated by $\mu$-opioid receptor in the rat spinal cord (Hayashida *et al.* 2003). LF enhanced analgesic action of morphine synergistically via nitric oxide synthesis (Hayashida *et al.* 2003). LF showed opioid-mediated suppressive effect on distress induced by maternal separation in rat pups (Takeuchi *et al.* 2003).

## Development of an enteric-coated LF tablet

In newborn babies, LF and other components of mother's milk go directly through the stomach into the intestinal lumen. In adults, orally administered LF is susceptible to the peptic digestion in the stomach. An enteric-coated LF tablet has been developed for the delivery of an intact molecule of LF onto the receptor in the intestine. In this formulation LF molecules are protected from the proteolytic digestion in the stomach since the tablet is coated by an acid-resistant material, which dissolves easily in a neutral pH condition in the intestine. Each tablet contains 50–100 mg of bovine LF. The tablet is stable for more than 2 years at room temperature since LF powder is formulated under an extremely dry condition and enteric-coated.

Thus, an intact LF molecule can bind to the receptor to show the multi-potent biological activities. The precise mechanism of action after the binding of LF to the receptor is still to be investigated. As is shown later, LF molecules could be absorbed effectively into the systemic circulation when administered orally in the enteric-coated formulation via receptor-mediated transcytosis to reach to each target organ to show various biological activities by a so-called 'Swiss Knife Model' as proposed by Prof. Shimazaki. An alternative mechanism in which LF signals on the intestinal immune system in a so-called 'Billiard Model' might also be possible.

LF enhances an anti-infective activity of antibiotics. In the experiment described by Miyazaki and his collaborators (Miyazaki *et al.* 1991), it was demonstrated that 5 mice out of 6 mice infected by Klebsiella pneumonia were dead without a treatment by Cephpodoxim. When LF was co-administered with the antibiotics, all mice survived even at the non-effective dose of LF used alone. We could determine the minimum effective dose of LF using this infection model.

The effective dose of Cephpodoxim for the 50% survival was decreased in the presence of LF. The minimum effective dose of LF was 0.05 mg per mouse. In human, therefore, the minimum effective dose was calculated to be 150 mg for the 60 kg body weight.

Dr Harada *et al.* have reported the transport of colostral components into cerebrospinal fluid via serum in neonatal pigs (Harada *et al.* 1999) and of latoferrin from the intestinal lumen into the bile via the blood in piglets (Harada *et al*, 1999). But in human, it has been unsuccessful to detect LF in the blood after the oral administration of even 20 grams of bovine LF as reported by Dr Tsuda.

Recently, Prof. Harada could detect bovine LF in human blood for the first time using the enteric-coated

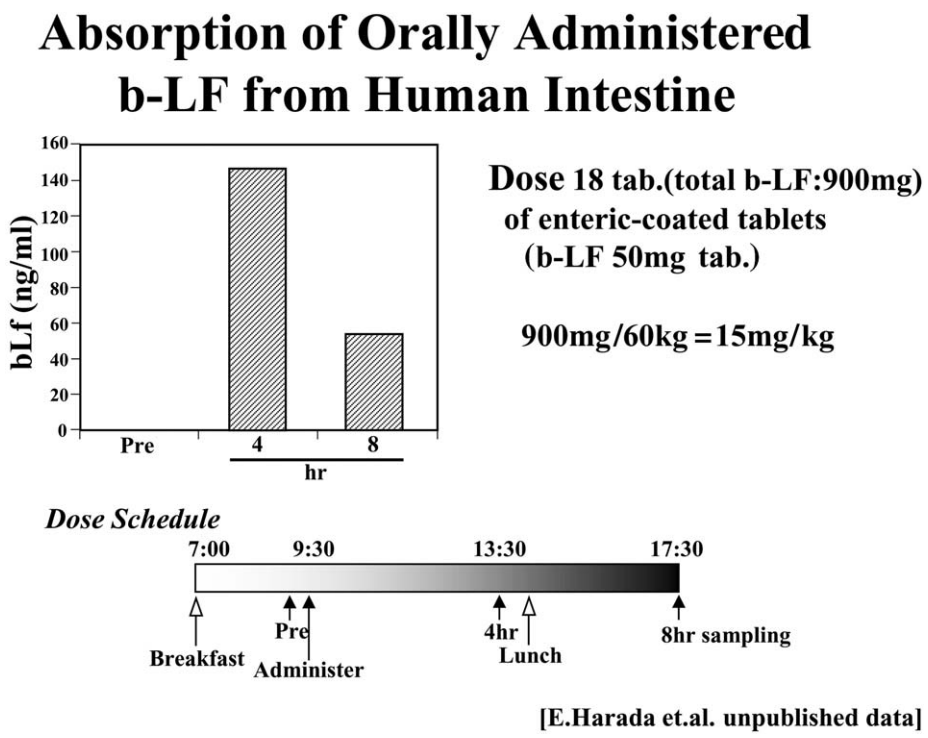



*Fig. 1.* Absorption of orally administered b-LF from human intestine.

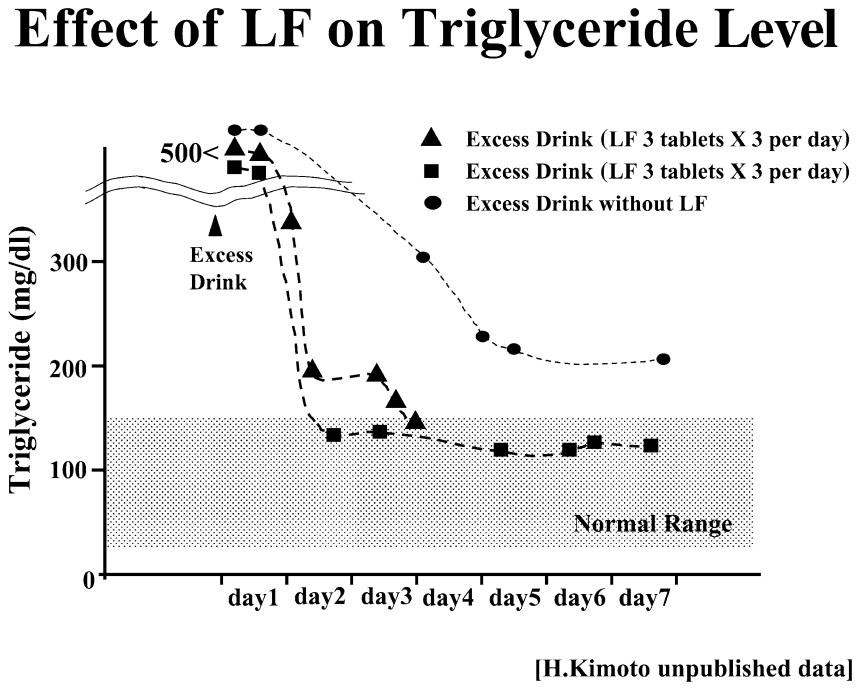
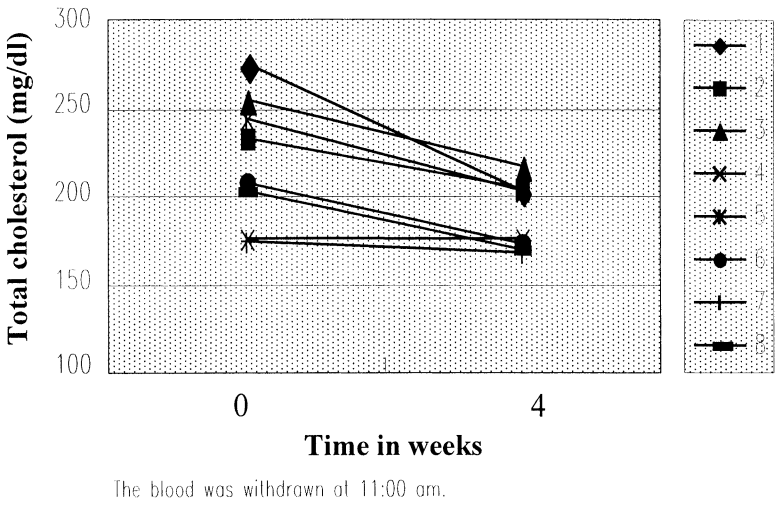



*Fig. 2.* Effect of LF on triglyceride level.

# Change in Total Cholesterol after 4 weeks

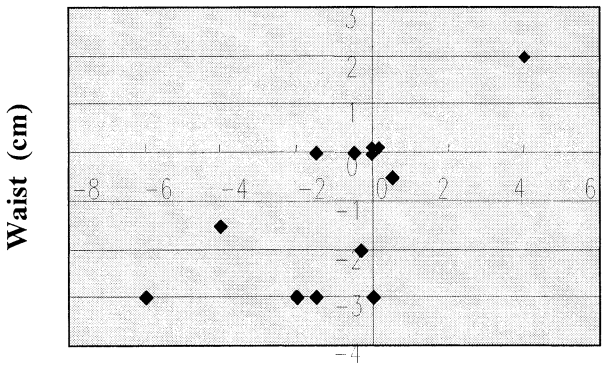



The blood was withdrawn at 11:00 am.

**[H.Kimoto .unpublished data.]**

*Fig. 3.* Change in total cholesterol level.

# Change of Body Weight and Waist




* 3-9 tablets per day of the enteric-coated b-LF for 1.2 months
* No special guidance about meal and exercise was given.
* In one case, the b.w. increased as a result of a good appetite.

**[H.Kimoto .unpublished data.]**

*Fig. 4.* Change of body weight and waist.

LF tablets after the oral administration of only 900mg of b-LF (Figure 1).

## Preliminary clinical results for the improvement of the lipid metabolism

Recently, Dr Hiroshi Kimoto of the Nagatsukai Saitoh Hospital, Chiba, Japan observed preliminary, but very interesting clinical results on the control of the lipid metabolism by an oral administration of the enteric-coated bovine lactoferrin tablets (Kimoto 2003).

The beneficial effects observed clinically by Dr. Kimoto are as follows:

(1) Rapid decrease of serum triglyceride in blood
(2) Significant hypocholesterolemic activity
(3) Stimulation of basal metabolic rate
(4) Reduction of body weight

The level of triglyceride in serum goes high up to the level of more than 500 mg per deciliter after excess drinking (Figure 2). The level of triglyceride decreased gradually to the level of still higher than the normal range even after 7 days. By taking 3 tablets at once, three times a day (total 450 mg of LF), the level decreased rapidly and reached to the normal range after 1–2 days.

Although the mechanism of LF action on the lipid metabolism needs further investigation, this rapid decrease of triglyceride level led us to speculate that LF switches on fat burning, leading to the stimulation of the basal metabolic rate as explained later.

The remarkable effect of the enteric-coated LF tablet as shown in Figure 2 led Dr Kimoto to study other clinical efficacies using the staffs of his hospital, who are mainly nurses, as volunteers. After taking 9 tablets a day for 4 weeks, the total cholesterol decreased in 7 cases out of 8 volunteers, suggesting hypercholesterolemia could be improved by this treatment (Figure 3).

Dr Kimoto studied on the possible stimulation of the basal metabolic rate by the enteric-coated LF tablet. The basal body temperature at rising seemed to shift slightly higher, and the body temperature 1 hour after meal also seemed to shift higher, suggesting the stimulation of the basal metabolic rate by taking the enteric-coated LF tablet.

If the basal metabolic rate is really stimulated by LF, the enteric-coated LF tablet may help dieting without any special guidance for foods and exercise. As is shown in Figure 4, the weight loss of 4–6 kg in a short period of 1–2 months was observed in some cases by taking 3–9 tablets a day. The reduction of 2–3 cm of the waist was seen in 5 cases out of 13 volunteers.

The results provide a speculation that lactoferrin may stimulate the uncoupled oxidation of fatty acids, resulting in the reduction of the body fat deposit. The hypocholesterolemic activity is probably due to the interruption of the enterohepatic circulation of bile acids by lactoferrin.

Although the enteric-coated LF tablet may be quite promising as a health supplement, but the potential of LF may lead to the development as a therapeutic agent for hyperlipidemia, hypercholesterolemia and obesity. There is no drug at present which can reduce both triglyceride and cholesterol like LF.

LF might also be a promising agent for the treatment of terminal cancer and arthritis since LF has both an anti-nociceptive and anti-stress activity and enhances synergistically an analgesic action of morphine.

Since LF has an anti-inflammatory activity, reducing TNF$\alpha$ and increasing IL-10, it might be useful for the treatment of colitis (Togawa *et al.* 2002).

LF is a unique multi-functional protein, which is effective for the treatment and prevention of many diseases. LF protects newborn babies from infection, stress and pain, which they encounter after birth. Furthermore, LF may switch on the lipid utilization as an energy source for life, reserving glucose as an energy source for brain development. In adults, we have to take LF in an enteric-coated formulation to enjoy such miraculous benefits.

## Acknowledgement

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