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Human Nutrition and Metabolism Research Communication

Orally Ingested Human Lactoferrin Is Digested and Secreted in the Upper Gastrointestinal Tract In Vivo in Women with Ileostomies¹

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ABSTRACT Lactoferrin (LF), a glycoprotein present in milk and other secretions, may provide a nutritional ingredient for the treatment of intestinal disorders associated with hyperpermeability, oxidative stress and inflammation. The aim of the study was to determine the survival rate of orally administered recombinant human lactoferrin (rhLF) in the upper gastrointestinal (GI) tract and in the small intestine in vivo in humans. Female ileostomy patients ($n = 8$; median age 44 (43–57) y) ingested a test beverage containing 5 g rhLF and collected full ileostomy output for 24 h. The passage of the test beverage was monitored using radio-opaque markers. Intact LF in ileal effluent was quantified by ELISA. The entire test beverage passed the small bowel during the 24-h ileal effluent collection period. LF was excreted at a low but constant rate [0.35 (0.28–0.47) μg LF/2 h]. Gastrointestinal passage of the test beverage and LF excretion over time in the ileal effluent were not correlated. In total, a median of 4 μg LF was excreted over the 24-h collection period, whereas 5 g rhLF was ingested. Hence, dietary rhLF is digested in the upper GI tract and does not reach the colon. Intact LF appearing in ileostomy effluent is likely to originate from an endogenous source. *J. Nutr.* 132: 2597–2600, 2002.

KEY WORDS: • lactoferrin • breakdown • digestion
• small intestine • ileostomy

Lactoferrin (LF)³ is a protein of the transferrin family. It is present in milk, saliva, tears, bile, blood plasma, mucosal and

genital secretions. LF is able to bind ferric iron (Fe^{3+}) with a high affinity at two different lobes. A number of physiologic effects have been attributed to LF. Previous studies have shown bactericidal (1–4), anti-inflammatory (5), antiviral (6–8) and antioxidative properties (9–11) of the LF molecule.

It has been suggested that LF has an anti-inflammatory potential. This effect seems to be mediated in part by attenuating tumor necrosis factor (TNF)- α response (12). TNF- α is involved in the pathogenesis of inflammatory bowel disease (IBD) (13). Hence, from a theoretical point of view, LF could potentially be used as an anti-inflammatory agent in the treatment of IBD. An anti-inflammatory effect would be expected after topical mucosal administration of the drug as in mesazaline and controlled ileal released budesonide therapy.

It is not known to what extent LF is degraded in the gastrointestinal (GI) tract in vivo in adults. Digestion may lessen the bioactivity of LF in the intestine. Oral LF supplementation may provide a nutritional tool to improve gut health especially the colon in humans. However, to exert any effects in the colon, LF has to survive upper GI transit. If it is digested in the stomach or in the small intestine, LF degradation should be prevented by the use of ileal release capsules or by administration via enemas when studying its effects in the colon.

We showed previously that LF is only partly degraded in the stomach in vivo in adults (14). The metabolic fate of orally ingested LF in the small intestine has not been investigated in vivo in adults previously. The present study was designed to determine the survival rate of orally administered recombinant human lactoferrin (rhLF) in the upper GI tract and in the small intestine in vivo in humans using the ileostomy model and to quantify the amounts of LF entering the colon in vivo.

MATERIALS AND METHODS

Study 1. Lactoferrin supplementation

Subjects. Female volunteers ($n = 8$) with an ileostomy [median age 44 (43–57) y] participated in the study. In all women, a total colectomy was performed at least 3 y before participation, enabling a steady-state situation after the initial adaptation phase to the colectomy. Women with Crohn's disease were permitted to participate in the study only if they had been in remission during the previous 3 y and if < 7.5% of the entire ileum was removed during previous surgery. None of the women were taking any medication or had any known abnormalities of the digestive system apart from the absence of the colon. All of the women were informed about the aims and the protocol of the study orally and in writing and they signed a written informed consent before participation. The study was approved by the Ethics Committee of the University Hospital Maastricht (Maastricht, The Netherlands).

Dietary restriction. Subjects were not allowed to consume any dairy products on the day before and on the day of the investigation to avoid dietary LF intake. They had to consume a standardized diet on each occasion. During breakfast at 0800 h and during lunch at 1300 h on the day before testing, they could consume bread rolls of any kind and nondairy beverages ad libitum. Dinner at 1800 h was a

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³ Abbreviations used: GI, gastrointestinal; IBD, inflammatory bowel disease; LF, lactoferrin; rhLF, recombinant human lactoferrin; TNF, tumor necrosis factor.

prepared meal, consisting mainly of chow mein (Dinner 2 today, Bami Goreng, Honig, Koog a/d Zaan, The Netherlands). On the day of testing, the women were not allowed to consume any food until breakfast at 1000 h. Subsequently, the diet was the same as described for the day before testing.

Protocol. After an overnight fast, subjects were admitted to the laboratory at 0800 h. They drank a test beverage containing 5 g rhLF (produced by *Aspergillus awamori*, Agennix, Houston, TX) dissolved in 0.10 L demineralized water. Immediately after ingestion of the test beverage, the stoma effluent bag was discarded as performed routinely. During the following 14 h, all stoma effluent was collected by replacing the stoma bag at 2-h intervals. Each stoma bag was placed separately in a small plastic airtight box on dry ice. Stoma effluent was collected every 2 h during the daytime until 2200 h. Subsequently, the women collected ileal effluent at 0300 and 0800 h.

Twenty radio-opaque plastic pellets (Sitzmarks Ø 2 mm, Konsyl Pharmaceuticals Fort Worth, TX) were added to the test beverage of each woman for a total of 160 pellets. These inert pellets can not be absorbed from the lumen and hence provide a marker for test beverage recovery in the ileal effluent.

Measurements. After collection, each ileostomy effluent sample was weighed to determine each 2-h and total 24-h ileal effluent production. All samples were X-rayed to count the number of radio-opaque pellets in each sample, which reflects the GI transit of the test beverage. In the ileostomy effluent samples, the amount of intact LF was determined by ELISA (Lactof-EIA, Biotech, Oxirisresearch, Portland, OR). The CV of repeated measurements of this assay was < 6% as measured in spiked ileal effluent samples. The sensitivity of the assay was 1 µg/L. Data were expressed as LF excretion over 2-h periods.

Study 2. Basal endogenous lactoferrin levels

In an additional in vivo pilot experiment, the endogenous LF excretion from the small intestine was investigated. On a separate day, four of the above-mentioned women collected ileal effluent during the last 5 h of the night. Emptying of the ileostomy bag at ~0300 h is common practice for most ileostomy patients. Ileal effluent collection took place from ~0300 to 0800 h. On the day before this collection, subjects consumed the same diet as during the first in vivo study. In a subsample of these ileal effluent samples, the LF concentration was measured by ELISA as described above.

Statistics. The amount of intact LF excreted in the ileal effluent during the 24-h period was expressed as LF excretion over 2-h intervals. Differences were assessed using the Wilcoxon signed rank test. Differences with $P < 0.05$ were considered significant. The median (10th–90th percentiles) ileal effluent production and LF excretion at each time point were calculated and presented as Box-and-Whisker displays.

TABLE 1

Excretion of radio-opaque pellets in the ileal effluent as an indicator of test beverage recovery after passage through the gastrointestinal tract of female ileostomists¹

Time of ileal effluent collection, h	Cumulative radio-opaque pellets recovered, n
0–2	0
2–4	32
4–6	72
6–8	108
8–10	147
10–12	150
12–14	154
14–19	157
19–24	0

¹ Eight women ingested a test beverage containing 20 radio-opaque pellets for a total of 160 pellets.

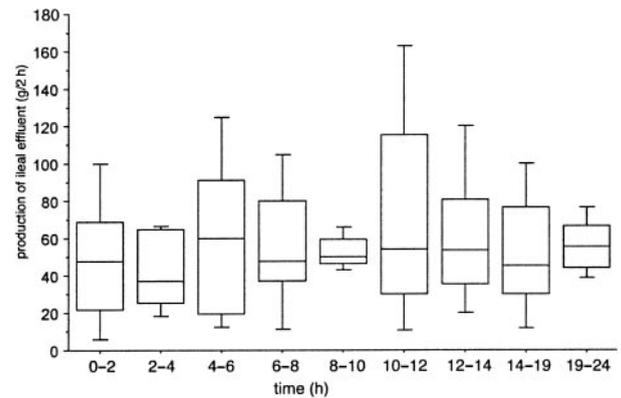


FIGURE 1 Ileal effluent production (24-h) of eight female ileostomists after ingestion of 5 g recombinant human lactoferrin. Medians (25th–75th percentiles) are displayed in boxes, 10th–90th percentiles are displayed in whiskers. Ileal effluent was produced at a constant rate during the collection period.

RESULTS

Study 1. Lactoferrin supplementation

Of the 160 total plastic radio-opaque pellets ingested with the test beverage (pooled over all experiments), 157 were excreted in the ileal effluents over the 24-h collection period. During the first 2 h after ingestion, no pellets were excreted. In the ileal effluent samples collected from 4 to 10 h after ingestion of the test beverage, 147 pellets were emptied into the stoma bags, whereas only 10 were excreted into the bags during the following 9 h. During the last 5-h collection period, no radio-opaque pellets were excreted (Table 1).

Ileal effluent production did not differ among any of the 2-h collection periods. The median total ileal effluent production over the 24-h collection period was 616 (456–865) g (Fig. 1).

The LF content of the ileal effluent samples did not differ among any of the samples. The median 2-h LF excretion was 0.35 (0.28–0.47) µg (Fig. 2). In total, a median of 4.01

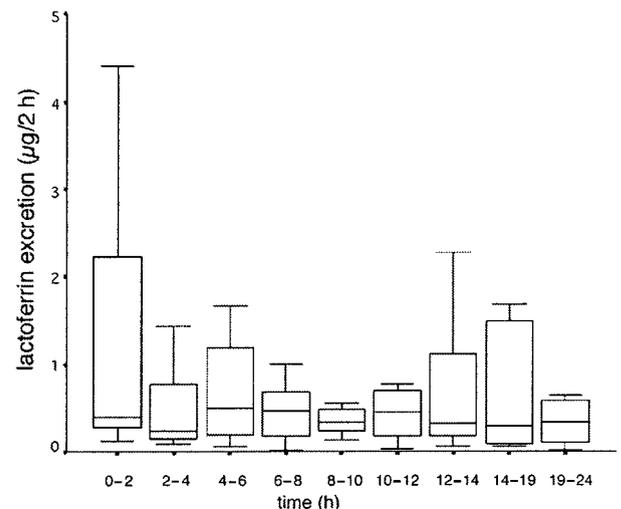


FIGURE 2 Ileal lactoferrin excretion (24-h) of eight female ileostomists after ingestion of 5 g recombinant human lactoferrin. Medians (25th–75th percentiles) are displayed in boxes, 10th–90th percentiles are displayed in whiskers. Low concentrations of recombinant or native human lactoferrin appeared in the ileal effluent at a constant rate.

(2.75–9.97) μg LF was excreted over the 24-h period by each woman (Table 2). The excretion of LF and the recovery of the radio-opaque pellets were not correlated.

Study 2. Basal endogenous lactoferrin levels

In the absence of LF ingestion, the 5-h ileal effluents of the four ileostomy patients contained 33 (24–52) μg LF/L ileostomy fluid. In the LF supplementation study, these subjects had a median 5-h ileal effluent LF concentration of 7 (6–9) $\mu\text{g}/\text{L}$.

DISCUSSION

Small amounts of intact LF appeared in the ileostomy effluent after oral ingestion of a LF-containing solution. The LF was excreted at a constant rate over the 24-h collection period. The recovery of the transit markers of the test beverage and LF excretion in the ileal effluent were not correlated.

Most if not all of the LF present in bile and intestinal secretions originates from specific granules of activated neutrophils (15–17). Plasma LF is secreted through bile into the intestine (18). In healthy adults, biliary LF is ~ 1 mg/L, and is ~ 3 mg/L in patients with IBD (17). Additionally, fecal LF is used as a diagnostic marker for immune system activation and hence, disease activity, in IBD patients (19). In the feces of healthy volunteers with an intact GI tract, LF excretion was 210 $\mu\text{g}/\text{d}$, whereas patients with inactive ulcerative colitis or inactive Crohn's disease excreted ~ 5 –6 mg/d (19). In the present study we found a median LF production of 4 $\mu\text{g}/\text{d}$ in ileostomists. The discrepancy between these observations can be explained by the hypothesis that the major part of LF in the GI tract originates from leukocytes in the colon. This seems to be a plausible conclusion because leukocytes are the major source of LF. We propose that the LF excreted in the ileal effluent may have originated from an endogenous source, not from the diet.

This was confirmed by the second in vivo study in which the women did not ingest LF, yet the ileal effluent samples had the same or higher LF concentrations than the samples collected over the same time period during the LF supplementation study. The effluent LF concentrations in the two studies did not differ significantly. Although only four subjects participated in the second study, we conclude that the LF detected in ileal effluents in both studies originated from an endogenous source.

The observation that orally ingested LF does not survive

passage through the small bowel confirms the findings of a LF breakdown study in the small bowel in rats (20). In that study, it was shown that during GI digestion of bovine LF, smaller fragments that exert biological activity against fungi are formed (21) and bacteria (3). This implies that although LF is digested completely in the upper GI tract and in the small intestine, oral LF ingestion may exert effects at any site in the GI tract through the action of LF fragments. We could not study the appearance of these fragments in the ileal effluent because of technical limitations in the quantitative analysis of LF fragments in the complex ileal effluent-matrix. Moreover, the present study does not exclude any biological activity of intact LF in the upper GI tract before degradation.

The rate of radio-opaque pellet excretion clearly showed that the test beverage was emptied from the small intestine within the 24-h ileal effluent collection period. This confirms the validity of the ileostomy model for studying GI survival of nutrients as we did in this study. Incomplete recovery of the markers was observed on two occasions. The omission of two markers in the ileal effluent of one volunteer and of one marker in another volunteer may have been caused by a solid particle that was retained temporarily in an intestinal fold. We conclude that the test beverage was completely emptied from the small intestine during the 24-h collection period.

The ileostomy model was used because it offered a unique chance to collect complete ileal effluent after the full transit of the test beverage through the upper GI tract. Hence, the bioavailability of any nutrient from the diet to the colon can be assessed. It is a well accepted method with which to study the digestion of nutrients and extrapolate the findings to subjects with an intact GI tract because the ileostomy patients participating in the present study have an intact upper digestive system.

A prerequisite for this technique of studying LF digestion in the GI tract in ileostomists is the stability of LF ex vivo after emptying into the ileostoma bag. LF may stay in the bag for a maximum of 2 h. Digestion may continue in the bag. Therefore we studied ex vivo LF degradation in ileal effluent. We did not observe any LF degradation during 2 h of incubation in fresh ileal effluent at 37°C (data not shown). This is consistent with a previous report in which no degradation of LF was observed during 2 h of incubation at 37°C in feces of healthy volunteers (19).

We studied digestion of recombinant human LF, produced in *A. awamori*. From three-dimensional structure comparisons it was shown that this type of rhLF has the same three-dimensional structure as the native human LF present in human breast milk (22). Dynamic properties of recombinant and native human LF are also similar. The only difference between the two LF forms is the composition of the glycans attached to the molecule due to the fungal system in which the rhLF is produced (23). The ELISA method used for quantification of intact LF present in the ileal effluent detected native human as well as rhLF.

Iron binding was shown previously to affect the stability of the LF molecule. Iron-saturated LF (holoLF) is more resistant to degradation than iron-free LF (apoLF) (24,25). The rhLF studied in the present study was free of iron. This is in contrast with studies using apoLF of animal origin in the natural state, which has an iron saturation of 15–20% (26). Therefore, the rhLF used in the present study may be more susceptible to GI degradation than LF of animal origin.

We showed that rhLF is completely degraded during passage through the stomach and the small intestine in vivo in humans. Intact LF entering the colon is unlikely to originate from an exogenous, dietary source of LF. This LF probably

TABLE 2

Lactoferrin excretion in the ileal effluent after oral ingestion of 5 g recombinant human lactoferrin in female ileostomists¹

Time of ileal effluent collection, h	Cumulative lactoferrin in ileal effluent, μg
0–2	0.39 (0.27–1.37)
2–4	0.61 (0.42–2.02)
4–6	0.89 (0.73–2.97)
6–8	1.34 (0.97–3.53)
8–10	1.82 (1.18–3.88)
10–12	1.99 (1.63–4.61)
12–14	2.22 (2.02–5.26)
14–19	2.95 (2.24–8.77)
19–24	4.01 (2.75–9.97)

¹ Values are medians (25th–75th percentiles), $n = 8$.

originates from granules of activated neutrophils in the upper GI tract. Therefore, if oral administration of LF does exert an effect in the colon, LF should be encapsulated for ileal release to prevent its degradation in the GI before it enters the colon.

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