Copper Intestinal Absorption in the Rat: Effect of Free Fatty Acids and Triglycerides (43984)

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Abstract. **The absorption of some minerals has been shown to be affected by the presence of unhydrolyzed dietary triglycerides and free fatty acids generated from their partial hydrolysis. Since copper (Cu) can form poorly soluble soaps with long-chain fatty acids, we examined whether the uptake of Cu from the intestinal lumen is altered by the presence of fatty acids and triglycerides using an *in vivo* jejunal perfusion procedure. Long-chain fatty acids palmitate and stearate at 1.0 m*M* reduced Cu absorption rates compared with infusates without either fatty acid or triglycerides (means ± SEM, controls: 104.4 ± 8.8 pmole/min × cm vs palmitate: 12.5 ± 17.6, $P <$ 0.01; stearate: 37.2 ± 25.6, $P < 0.05$). Medium chain free fatty acids had no effect on Cu absorption (caprylate: 90.6 ± 14.9, not significant; caproate: 69.5 ± 14.2, not significant). Similarly, neither an emulsion of medium chain nor long-chain triglycerides at a total 1.0 or 2.5 m*M* concentration altered Cu absorption. The inhibitory effect of palmitate and stearate on Cu absorption was accompanied by a reduction in lumen-to-mucosa water influx (controls: 5.33 ± 0.26 μl/min × cm vs palmitate: 3.20 ± 0.70, P $<$ 0.01; stearate: 3.36 ± 0.52, $P < 0.01$). The data are consistent with a potential impairment of Cu intestinal absorption by long-chain free fatty acids which may accumulate in the jejunum following excessive fat intake and/or lipid malabsorption.**

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The absorption of essential trace elements is modulated by several dietary components. Protein content (1, 2) is generally an agonist in this process, while dietary fiber (3–6) tends to bind many di- and trivalent cations, and reduce their bioavailability. Less attention has been focused on dietary fat as a modifier of mineral absorption, although it is known that the binding of cations, as well as their fractional distribution among hydro- and liposoluble phases, is greatly influenced by lipid content (7). During the intestinal digestion of triglycerides, a release of free fatty acids and their incorporation into micelles occurs (8, 9). However, the fatty acids can still interact with certain di- and trivalent cations. Most of the salts of long-chain fatty acids, other than those of sodium and potassium, are poorly soluble in an aqueous medium. The solubility product of divalent and trivalent metal salts of long-chain fatty acids is small enough to result in minimal ionization and possible precipitation of these organometalic products on solid undigestible particles which can act as phase transition nuclei. In addition, alkaline salts of fatty acids are also surface active agents and can possibly interact with the mucosal brush border, thus modifying trace element absorption.

This study was aimed at testing the hypothesis that either medium chain or long-chain free fatty acids could interfere with the absorption of Cu and that unhydrolyzed triglyceride mixtures could also alter the luminal phase of Cu absorption.

Materials and Methods

Experimental Procedure. Experiments involved the perfusion of 20–30 cm long jejunal segments in 80- to 100-g Sprague-Dawley male rats (Zivic-Miller

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Labs., Zeleniople, PA). The rodents were used at least 48 hr after acclimatization to the animal housing facility. They were fed Purina Rat Chow (Ralston Purina Co., St. Louis, MO) and tap water *ad libitum*. Food, but not water, was withheld the night prior to the experimental procedure. The perfusion technique used has been extensively described in earlier publications (2, 10). The perfusion pumping rate was maintained at 0.18–0.20 ml/min to avoid distension of the perfused segment. The solutions were warmed so that they would enter the proximal end of the jejunal section at the rat body temperature (37°C). Animals were anesthetized with ip urethane (1.3 g/kg) and maintained over a heating pad for the duration of the experiment. The perfusions consisted of an initial period of equibration for 1 hr, followed by eight 15-min collections of effluent fractions over the subsequent 2 hr. The rats were then exsanguinated from the abdominal aorta, while still under anesthesia. The jejunal segment was removed, rinsed with cold saline, its length measured under moderate tension (3 g), and weighed. Animal experimentation followed the prescriptions of the *Guide for the Use and Care of Laboratory Animals* (NRC, 1985). The protocol was approved by the Institutional Animal Care and Utilization Committee.

Laboratory Determinations. The computation of Cu absorption rates, net water absorption, and unidirectional water fluxes was performed with the algorithms previously published (2). In summary, the rate of net water absorption was estimated from the difference between the weight of solution pumped per unit of time and the amount collected during the same period from the distal end of the canulated jejunal segment. Water movement from lumen-to-serosa was estimated from the disappearance of tritiated water from perfusates added at a concentration of approximately 74 kBq/l (ICN Biomedical Inc., Costa Mesa, CA). Serosa-to-mucosa water efflux was calculated by difference between water influx and net water absorption. Disappearance of Cu from the intestinal lumen, corrected for water volume changes, was taken as the index of Cu absorption. In all perfusions Cu was added as the nitrate at a concentration of 0.031 m*M* (2 mg/l) in an isotonic glycerol solution buffered with 5 m*M* Pipes-tris pH 6.8 (Table I). This concentration of Cu in the infusates was consistent with usual dietary levels of rodents and humans. L-histidine was routinely included in all solutions at a 2:1 ratio to Cu to prevent insolubilization of Cu by free fatty acids. Prior to its assay, Cu was chelated by the addition of approximately 100 mg of disodium EDTA to each fractional collection, and quantitated by atomic absorption spectroscopy (Varian SpectrAA 10, Sunnyvale, CA) against external standards (Fisher Scientific, Fair Lawn NJ). The osmolality of the solutions was determined with a vapor pressure osmometer (Wescor Model 5500, Ogden, UT) calibrated with certified standards, and maintained at 290 ± 10 mOsm/kg. The number of animals used is indicated in figures and tables.

Preparation of Lipid Substrates. The free fatty acids, caprylate (C8), caproate (C10), palmitate (C16), and stearate (C18), were prepared as the water-soluble potassium salts in stocks of 0.1 *M* concentration. The solution of medium-chain triglycerides (MCT Oil; Bristol Myers Squibb, Nutr. Div., Evansville, IN) was emulsified with L-α-phosphatidylcholine (α-lecithin) as indicated in Table I. All chemicals were purchased from Sigma Chemical Co. (St. Louis, MO). A molecular weight of 570 kDa was assigned to MCT Oil. As a source of long-chain triglycerides (LCT) a soybean oil emulsion was used (Intralipid; KabiVitrum, Alameda, CA) and considered to have a mean molecular weight of 840 kDa. This product already contains α-lecithin. In perfusion experiments with LCT and MCT, the control solution also contained α-lecithin.

Statistical Analysis. Data in the figures and ta-

Table I. Composition of the Solutions Perfused in the Experiments to Determine the Effects of Free FA and TG on Copper Intestinal Absorption

	Control	Palmitate	Stearate	Caprylate	Caproate	MCT Oil	LC TG
Cu (as nitrate)	0.031	0.031	0.031	0.031	0.031	0.031	0.031
L-Histidine	0.062	0.062	0.062	0.062	0.062	0.062	0.62
Glycerol	280	280	280	280	280	280	280
Pipes-Tris (pH 6.8)	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Palmitate (K salt)	—	1.0	—	—	—	—	—
Stearate (K salt)	—	—	1.0	—	—	—	—
Caprylate (K salt)	—	—	—	1.0	—	—	—
Caproate (K salt)	—	—	—	—	1.0	—	—
Medium-chain triglycerides (MCT Oil)	—	—	—	—	—	1.0/2.5	—
Long-chain triglycerides (Intralipid)	—	—	—	—	—	—	1.0/2.5
Phospholipids or lecithin (g/l)	—	—	—	—	—	12.0	12.0
Phenol red (mg/l)	20	20	20	20	20	20	20

Note. Concentrations are presented in millimolars, unless noted otherwise.

bles are presented as means ± SEM. The results were evaluated by analysis of variance (ANOVA). Differences between the experimental groups and the controls were discriminated by Dunnett's test (11). The threshold of significance was set at 0.05.

Results

Copper Absorption. In the presence of 1.0 m*M* of either the long-chain fatty acids palmitate or stearate, there was a reduction in the absorption rates of Cu (Fig. 1). The values obtained with either of the two long-chain fatty acids could not be differentiated. In contrast, when the medium-chain fatty acids caprylate or caproate were perfused no alterations in Cu absorption were detected. Emulsions containing either 1.0 or 2.5 m*M* of the medium chain triglyceride mixture MCT Oil or long-chain triglycerides (Intralipid) produced no changes in Cu absorption rates (Table II).

Water Absorption. Net water absorption measured in the course of Cu perfusions was unaffected by the presence of free fatty acids or triglycerides (Table III). However, changes in unidirectional water movement paralleled Cu absorption. In the presence of either 1.0 m*M* palmitate or stearate, a very significant decline in both the lumen-to-serosa water inflow and serosa-to-mucosa water efflux was observed. In contrast, the addition of similar concentrations of caprylate or caproate produced no alterations in fluid transport rates.

Cu and Fluid Transport Interactions. In the absence of free fatty acids or triglycerides there was a positive correlation between both net water absorption and lumen-to-serosa water influx and Cu absorption (Fig. 2A). A comparable correlation held for net water absorption and the rates of Cu removal from the lumen (not shown). There was also a very significant positive correlation between water influx and Cu absorption in the presence of either 1 m*M* palmitate or stearate. These findings were consistent with the notion that the jejunal absorption of Cu occurs largely by bulk transport, at least when this element is perfused at 0.031 m*M* concentration. In the presence of long-chain fatty acids the slope of the regression was greater than in their absence (4.386 vs 1.606). An examination of the values corresponding to water influx rates, which are determined by the disappearance of tritiated water from the lumen, indicated that a substantial rate of water influx, approximately 3 μl/min × cm, was the threshold required for Cu removal from the lumen, both in the absence or the presence of long-chain free fatty acids. When the rates of water influx in the presence of either MCT or LCT were plotted against Cu absorption rates, a significant positive correlation was obtained for the data corresponding to MCT emulsions (Fig. 2B). In contrast, the water influx rate did not correlate with Cu uptake, when the latter was perfused with 1.0 or 2.5 m*M* of LCT emulsion. Similarly, the uptake of Cu, in the presence of either medium-chain fatty acids (caprylate or caproate) or long-chain fatty acids (palmitate or stearate), did not correlate with lumen-to-serosa water influx.

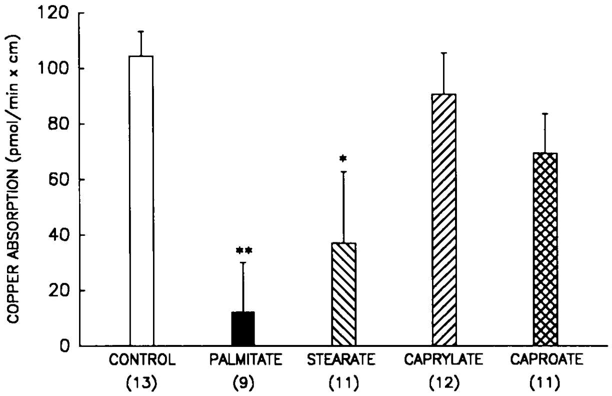

Figure 1. Jejunal copper absorption rates in the absence and the presence of 1.0 m*M* of either of the fatty acids indicated in the legend. The error bars denote the SEM. The asterisks represent the significance of the differences of the respective additives compared to an infusate containing no fatty acids. The numbers in parentheses correspond to the number of rats tested for each condition. **P* < 0.05; ***P* < 0.01 against controls.

Table II. Cu Absorption Rates in the Presence of MCT Oil or Intralipid Emulsions

	Concentration of emulsion (m*M*)		
	0	1.0	2.5
Controls	85.3 ± 7.7	—	—
(*n*)	(18)		
MCT Oil	—	81.2 ± 11.7	79.8 ± 6.6
(*n*)	—	(9)	(10)
Intralipid	—	104.1 ± 9.7	102.5 ± 11.3
(*n*)	—	(10)	(5)

Note. The data are expressed as means ± SEM.

Discussion

This study indicates that a relative excess of long-chain free fatty acids, predominant components of adult dietary fat intake, inhibits the jejunal absorption of Cu (Fig. 1). The chain length may be of primary importance, since medium-chain fatty acids, such as caprylate and caproate had no effect on the absorption of that element. This finding is consistent with the progressive insolubility in water and in hydrophobic solvents of Cu salts of fatty acids with increasing chain length (12).

In our experiments, both the water influx and efflux were reduced in the presence of either palmitate or stearate. This finding could be linked to one of the following possibilities: (i) an interaction between the slightly soluble Cu salt of the two long-chain fatty acids and the negatively charged brush border glycocalyx, or (ii) a decreased fluid movement largely due to

Table III. Unidirectional Water Fluxes during Perfusions of Copper in the Presence of Either Long- or Short-chain Triglycerides, or Free Fatty Acids

	Net water absorption (μl/min × cm)	Water influx (μl/min × cm)	Water efflux (μl/min × cm)
Controls	2.50 ± 0.20 (26)	5.33 ± 0.26 (26)	2.84 ± 0.17 (26)
1.0 m*M* long-chain triglycerides (Intralipid)	1.93 ± 0.19 (10)	4.88 ± 0.52 (10)	2.67 ± 0.41 (10)
2.5 m*M* long-chain triglycerides (Intralipid)	2.56 ± 0.22 (5)	5.22 ± 0.27 (5)	2.67 ± 0.45 (5)
1.0 m*M* medium-chain triglycerides (MCT Oil)	1.98 ± 0.30 (9)	5.21 ± 0.22 (9)	3.23 ± 0.26 (9)
2.5 m*M* medium-chain triglycerides (MCT Oil)	2.62 ± 0.30 (10)	5.11 ± 0.21 (10)	2.49 ± 0.27 (10)
Palmitate 1.0 m*M*	1.89 ± 0.23 (6)	3.20 ± 0.70[a] (6)	1.31 ± 0.51[a] (6)
Stearate 1.0 m*M*	1.92 ± 0.32 (10)	3.36 ± 0.52[a] (10)	1.44 ± 0.45[a] (10)
Caprylate 1.0 m*M*	2.73 ± 0.29 (12)	5.32 ± 0.27 (12)	2.59 ± 0.24 (12)
Caproate 1.0 m*M*	2.50 ± 0.26 (11)	4.87 ± 0.24 (11)	2.37 ± 0.19 (11)

Note. Data are means ± SEM. Number of animals is presented in parentheses.
[a] $P < 0.01$ versus controls.

the presence of free fatty acids. Although inhibition of water absorption in the ileum by free fatty acids has been described earlier (13), the permeability and transport characteristics of the jejunum are different, and it cannot be directly determined whether the effect observed in this study was due to the Cu salt of the free fatty acid or to the free fatty acids by themselves. Free fatty acids were present in the perfusates at an approximately 30:1 ratio to Cu; however, this excess, *per se*, is not necessarily inhibitory, in view of earlier results obtained during zinc-free fatty acid perfusions (2). In consequence, there is an apparent linkage among the presence of a long-chain fatty acid-Cu soap in the infusate, the reduction of water absorption, and the overall decrease of Cu removal from the jejunal segment.

In contrast to the free fatty acids, the unhydrolyzed triglycerides, whether made of medium- or long-chain fatty acids, altered neither Cu nor net water absorption. The unidirectional fluxes of water were similarly unchanged. The presence of phospholipids or lecithin in the concentrations used in the perfusion, either in the MCT Oil emulsion prepared in our laboratory for the perfusion, or present in the Intralipid used as a source of long-chain triglycerides (Table I), did not affect water movement. The correlation between Cu absorption and water transport is informative in showing that Cu, in the absence of either free fatty acids or triglycerides, behaved as a typical solute transported by diffusion across the jejunal mucosa (Fig. 2A). The steeper slope of the regression between Cu absorption and water influx in the presence of palmitate and stearate, compared with that obtained in their absence, suggests that other metal uptake mechanisms (i.e., mediated transport [14]) probably have a greater role in Cu absorption than bulk flow. Diffusion also appears to be the major factor involved in the transport of Cu in the presence of MCT (Fig. 2B). However, in the presence of unhydrolyzed LCT, the lack of correlation between Cu absorption and water influx mitigates against a preeminent role for bulk flow.

Long-chain free fatty acids such as palmitate and stearate have been found to have pharmacologic effects at the concentrations used in this experiment (15). Dietary lipids, monoglycerides, and free fatty acids generated during the normal process of fat digestion and absorption (8, 9) can change the permeability of the cellular membrane to solutes by interacting with the hydrophobic domain of the brush border barrier (16). In the suckling rat, there is a decline of hydrophobicity, an increase in phospholipid concentrations, and a reduction of long-chain fatty acid absorption rates as the lactation period progresses (17). In view of the results presented here, it could be inferred that the bioavailability of Cu, especially for infants and children, could greatly vary as a function of the digestibility of fats in either commercial formulae or cow's milk (18, 19). The concentrations of triglycerides and free fatty acids used in this study are comparable to those found in fluid dairy products.

Although Cu nutritional status of the rats was not assessed in this study, the consistency in supplier source and maintenance patterns made this factor a constant in the current comparison of potential luminal modulating variables. Future experiments may reveal

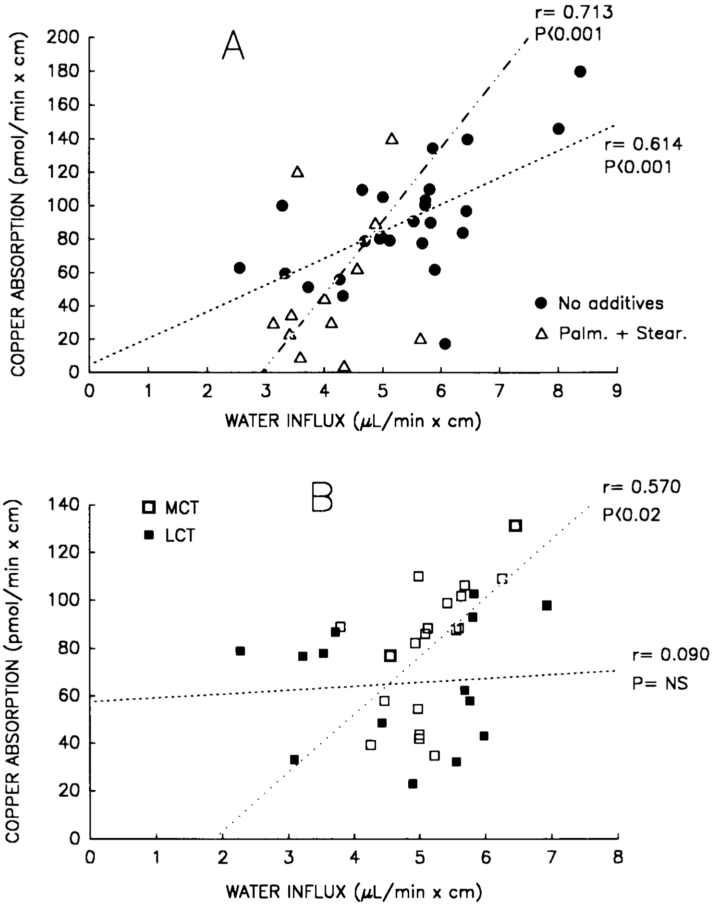

Figure 2. **(A)** Regression lines of jejunal copper absorption rates in the absence of additives (●) and in the presence of either 1 m*M* palmitate or stearate (△) against water influx. The correlation coefficient and their significance indicated in the graph correspond to 23 d.f. for no additives, and 19 d.f. for palmitate + stearate. **(B)** Regression lines of copper absorption rates in the presence of 1.0 m*M* of either medium chain triglycerides (□, MCT, ······) or long chain triglycerides (■, LCT, ------). The significance of the regressions is shown in the figure.

whether Cu sufficiency or alterations in mucosal metallothionein induction may play a role in long-chain fatty acid inhibition of Cu absorption.

There is fragmentary knowledge on the interactions between dietary fat and minerals. Steatorrhea of varied etiology has been associated with Cu malabsorption due to small bowel disease (20). Magnesium (21) and possibly calcium malabsorption (22) have also been observed as a result of steatorrhea. The formation of calcium soaps is known to occur during the feeding of free fatty acids. The ingestion of egg yolk, a rich source of lipids, has been shown to decrease the absorption of inorganic iron in rats, although this effect was not entirely clear in humans (23). In a clinical study, it was found that dietary polyunsaturated fatty acids had no effect on Cu retention, but reduced that of zinc and iron (24). Diabetic rats, fed a high-fat diet, had lower liver Cu concentrations than animals fed a high-carbohydrate diet (25). Work from our laboratory has revealed that a high-fat ingestion by rats becomes an aggravating stress factor in Cu deficiency, especially in combination with high fructose intake (26).

An additional linkage may exist between Cu assimilation, dietary fat, and lipid metabolism. Nutritional Cu deficiency has been associated with hypercholesterolemia, low HDL cholesterol, plasma triglycerides, and lipoproteins (27, 28). High-fat consumption entailing the ingestion of an excessive amount of fatty acids is prevalent in the United States. A related risk factor is high alcohol consumption, which alters lipid permeability of membranes and aggravates the sequelae of an insufficient Cu nutriture (29, 30). Furthermore, Cu deficiency more than doubles the rate of hepatic fatty acid and triglyceride synthesis (31), and the type of dietary fat affects the kind of cardiac hypertrophy associated with Cu deficiency in rats (32). In consequence, the information presented here on Cu intestinal absorption focuses on a hitherto overlooked possible consequence of a high-fat diet and/or lipid malabsorption as determinants of a deficient Cu nutritional status.

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