# SMALL INTESTINAL MUCOSAL FATTY ACID UPTAKE AND ESTERIFICATION IN INFANTS AND CHILDREN

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Supported in part by the United Order True Sisters of Ruth and the N.I.H. General Clinical Research Center Grant #RR-00123.

## SUMMARY

Oleic acid uptake and esterification in intact intestinal mucosa were studied in 14 infants and children with chronic non-specific diarrhea, but histologically normal small intestinal mucosal biopsies, using an in vitro technique. The uptake rate was 5.876 ± 1.942 nmol fatty acid/mg Nitrogen/minute and the esterification rate was 4.060 ± 1.010 nmol fatty acid/mg Nitrogen/minute, comparable to previous adult esterification studies. No effect of age on either esterification or uptake was present. Mucosal injury resulted in significant reductions in esterification ($p < 0.001$) and uptake ($p < 0.05$) compared to controls. Bile acid deficiencies led to reductions in mucosal esterification ($p < 0.05$) but not uptake.

## SPECULATION

The intestinal mucosal biopsy has been an invaluable tool in the study of the histopathology and ultrastructural pathology of human disease; it will become a valuable tool to study intestinal biochemistry, enzymology and physiology. The simple in vitro incubation technique described, with application to the study of fatty acid uptake and esterification and to other areas of mucosal metabolism, may allow delineation of new etiologies for chronic infantile diarrhea. Specific alterations in mucosal metabolism may be found associated with alterations in intestinal mucosa morphology or intraluminal conditions.

## INTRODUCTION

Peroral, non-invasive intestinal mucosa biopsy has proven useful in diagnosis, treatment and in understanding the pathophysiology of gastrointestinal diseases. The peroral biopsy technique allows histopathologic and ultrastructural study of mucosal changes during life which are otherwise impossible because the small intestinal mucosa is rapidly destroyed after death. The full value of the mucosal biopsy has not been exploited in the study of childhood intestinal disease because few attempts have been made to adapt modern methods of biochemistry, physiology and cell biology to the study of intestinal mucosa biopsy samples.

The advent of total parenteral alimentation has allowed the survival of certain cases of congenital steatorrhea, some of whom may have defects in the fatty acid uptake-resynthesis steps within the intestinal absorptive epithelium. Few human studies of intestine glyceride uptake and esterification have been performed; no studies have been undertaken in infants and children, and little is known about the developmental biology of triglyceride transport in the human intestinal mucosa (2,3,6,18).

This study is designed to determine whether fatty acid uptake and re-esterification are altered in the intestinal mucosa of certain infants with chronic diarrhea. The study was prompted by the need to evaluate intestinal glyceride uptake and synthesis in an infant with congenital steatorrhea, in whom clinical, biochemical and balance studies suggested the possibility of a defect of intestinal triglyceride resynthesis (5). The in vitro method which was developed quantitatively measures fatty acid uptake and esterification in whole Crosby-Kugler intestinal biopsy samples. The method should prove to be of value in the future investigation of developmental biology, biochemistry and enzymology of the human intestinal mucosa as well as in the search for congenital and acquired metabolic defects of intestinal triglyceride transport.

## MATERIALS AND METHODS

Patients were selected for study from children referred to the Gastroenterology Division with diarrhea of two months to five years duration. All studies were performed on the General Clinical Research Center. Approval for the study was obtained from the Institutional Committee on Human Investigation. Informed consent from the parent or guardian was obtained before the study. Diagnostic investigations including blood studies for assessment of hematologic, renal and hepatic function; urine for culture and catecholamine determination; sweat for chloride analysis; duodenal drainage for pancreatic enzyme assay and bacteriologic and parasitic examinations were performed. Stool was examined for pathologic bacteria and parasites and for reducing substances by Clinitest(R). Quantitative fecal fat was measured on 72 hour collections in all but 3 children.

Three groups of patients were studied. Group 1, chronic non-specific diarrhea, consisted of 14 patients referred to the Children's Hospital Medical Center by their personal physicians because of so-called chronic non-specific diarrhea. The referring diagnosis was frequently celiac disease. After thorough investigation, including diagnostic small intestinal biopsy, the children in this group were found to have histologically normal intestinal mucosas and no steatorrhea, although many were considered by their parents to have somewhat loose stools. On long term evaluation, all Group 1 patients have had normal growth and development.

Group 2, mucosal injury, included 5 children with protracted diarrhea, steatorrhea, and weight loss with histologic or ultrastructural evidence of definite mucosal injury. Quantitative stool collections were performed on four patients with the following stool weights and fat excretion per 24 hours: 1) 269.0 grams stool, 1.8 gram fat, 2) 152.9 grams stool, 5.5 grams fat, 3) 67.1 grams stool, 11.1 grams fat and 4) 104.6 grams stool, 7.9 grams fat (normal 50-100 grams stool, < 5 grams fat/24 hours). Intestinal mucosal biopsies demonstrated moderate to moderately severe jejunitis in which crypt height was more or less increased at the expense of villus height, but none had severe shortening of the villi. All had mild to moderate infiltration of the epithelium and lamina propria with lymphocytes and, in one case, plasma cells. The epithelial cells looked relatively normal in 2, an increased number of lysosomes was seen in 1, and reductions in the height of the epithelial cells were present in 2.

Group 3, children with accelerated bile acid turnover, was comprised of 3 children with diarrhea, steatorrhea, and growth failure. Fecal excretion of cholic-COOH-$^{14}$C in one patient was 31.5% of the orally administered dose after 24 hours and 57.8% after 72 hours (normal 4.8 ± 1.0% at 24 hours, 16 ± 3.6% at 72 hours) (1). The fractional turnover rate ($days^{-1}$) for intravenously administered cholic-COOH-$^{14}$C was 1.84 and 2.16 for the other two patients studied [normal adults 0.321 ± 0.12 (17)], and intraluminal bile salts with Lundh meal stimulation were reduced compared to age-matched controls, but not below the critical micellar concentration. No significant abnormalities of small intestinal mucosa histology were present.

## METHODS

After an overnight fast, a 1.8 mm twin port or 3.0 mm port Crosby-Kugler intestinal biopsy capsule was passed to the distal duodenum or jejunum† The capsule was fired and mucosa removed. A portion was frozen, a portion placed in formalin for routine histologic study, and, in selected cases, a portion prepared for electron microscopy. The remaining portion of mucosa was transported to the laboratory, placed in a pre-warmed 10 ml Erlenmeyer flask containing one milliliter of a modification of the incubation solution of Johnston and Borgstrom (8):

Krebs-Ringer phosphate (KRP) buffer without calcium or magnesium and 10.0 mM glucose (pH 6.3) gassed for 1 hour with 95% $O_2$-5% $CO_2$ containing:

1. 2.4 mM Na taurodeoxycholate (Calbiochem, Los Angeles, CA.)
2. 0.06 mM oleic-1-$^{14}$C, 40-60 mCi/mmol (New England Nuclear, Boston, MA.), purity verified in our laboratory by thin layer chromatography.
3. 0.54 mM oleic acid (Sigma, St. Louis, MO.)
4. 0.3 mM glycero-2-monopalmitate (Supelco, Bellefonte, PA.), purity verified in our laboratory by thin layer chromatography.

The flask was gassed for 1 minute with 95% $O_2$-5% $CO_2$ and incubated with shaking (40-45 oscillations/minute) at 37.0 ± 0.5°C for 8 minutes.

After incubation, the tissue was removed from the incubation flask, washed with iced KRP buffer in a petri dish and blotted dry. The tissue was homogenized and an aliquot removed for Kjeldahl nitrogen determination (15). The remaining homogenate was filtered, transferred to a separatory funnel, lipid standard (Sigma, St. Louis, MO.) added, and lipid extracted twice (4). The extract was dried, resuspended in 200 µl $CHCl_3$ and lipid classes separated on pre-coated 0.25 mm thickness silica gel 60 plates (E. Merck, Darmstadt, Germany) (13). Lipid spots were visualized with $I_2$ vapor, scraped into liquid scintillation vials, scintillation cocktail added and the radioactivity counted in a Packard Model 3375 Tri Carb Liquid Scintillation Spectrometer.

Preliminary studies in which duplicate specimens were incubated under similar conditions produced a coefficient of variation between samples of 7.82% (n=4). Incubation of duplicate specimens in varying concentrations of oleic acid to verify optimal incubation conditions resulted in reductions in esterification and uptake at concentrations below and above the concentrations used in the present study (n=10). Linear increases in fatty acid uptake and esterification rates were found at 4,6 and 8 minutes incubation time. Incubations longer than 8 minutes resulted in no increase in esterification or uptake. Mucosal integrity after incubation was verified in two patients by electron microscopy.

## CALCULATIONS

Fatty acid uptake was determined from total lipid present in all lipid classes separated by thin layer chromatography:

$$\text{Uptake} = \frac{\text{dpm (all lipid classes)/specific activity (dpm/µmol)}}{\text{mucosal nitrogen (mg)/incubation time (min)}}$$

Fatty acid esterification was determined by adding the total lipid present in the di- and triglyceride spots by thin layer chromatography:

$$\text{Esterification} = \frac{\text{dpm (di- and triglycerides)/specific activity (dpm/µmol)}}{\text{mucosal nitrogen (mg)/incubation time (min)}}$$

Percentage distribution of lipid classes was determined by thin layer chromatography.

## STATISTICS

Patient groups were evaluated statistically by the single classification of analysis of variance and Wilcoxon rank sum methods (14).

## RESULTS

Intestinal mucosal fatty acid uptake and esterification in the fourteen children in Group 1 were 5.88 ± 1.94 nmol/mg Nitrogen/minute and 4.06 ± 1.01 nmol/mg Nitrogen/minute, respectively (Table 1). The esterification-to-uptake ratio was 0.71 ± 0.14. There was no significant change in either uptake or esterification with age (Figure).

The effect of mucosal injury (Group 2) on mucosal fatty acid uptake and esterification was evaluated in five children. Significant reductions in uptake, 3.36 ± 1.16 nmol/mg Nitrogen/minute, esterification, 1.80 ± 0.47 nmol/mg Nitrogen/minute, and esterification/uptake ratio, 0.55 ± 0.08, were present when compared with Group 1 (Table 1).

The children with intraluminal bile acid deficiencies (Group 3) had significantly reduced esterification, 2.63 ± 0.62 nmol/mg Nitrogen/minute, compared with Group 1. No significant reductions in uptake or esterification/uptake ratios were present.

The distribution of fatty acid, diglyceride and triglyceride in mucosa in Groups 1,2 and 3 are shown in Table 2. Diglyceride content was significantly increased and triglyceride reduced in Group 2 compared with controls. No change was present in Group 3.

## DISCUSSION

Gastrointestinal triglyceride digestion and absorption is initiated when pancreatic lipase in the presence of bile salts hydrolyzes dietary fat to fatty acids and 2-monoglycerides. The intraluminal digestion products are passively absorbed into the intestinal epithelial cell and must then be resyn-

† Obtainable from Quinton Instruments, 3051 44th Avenue West, Seattle, Washington 98199

TABLE 1

SUMMARY OF MUCOSAL INCUBATION STUDIES IN INFANTS AND CHILDREN

| PATIENT GROUP | NUMBER | AGE | UPTAKE (NMOL/MGN/MIN) | ESTERIFICATION (NMOL/MGN/MIN) | ESTERIFICATION / UPTAKE |
|---|---|---|---|---|---|
| GROUP 1 (CONTROL) | 14 | 4MOS-14YRS | 5.88 ± 1.94 (SD) RANGE: 3.86-10.24 | 4.06 ± 1.01 2.77 - 6.08 | 0.71 ± 0.13 0.43-0.86 |
| GROUP 2 (MUCOSAL DISEASE) | 5 | 18MOS-12YRS | 3.36 ± 1.16 RANGE: 2.00-4.78 p < 0.05* | 1.80 ± 0.47 1.26 - 2.44 p < 0.001* | 0.55 ± 0.08 0.46-0.64 p < 0.05* |
| GROUP 3 (BILE ACID DIARRHEA) | 3 | 31MOS-5YRS | 3.38 ± 0.92 RANGE: 2.95-5.06 N.S.* | 2.63 ± 0.62 2.09 - 3.50 p < 0.05* | 0.70 ± 0.01 0.69 - 0.71 N.S.* |

*P VS GROUP 1

TABLE 2

DISTRIBUTION OF MUCOSAL LIPID (%)

| GROUP | FATTY ACID | DIGLYCERIDE | TRIGLYCERIDE |
|---|---|---|---|
| 1 | 21.2 ± 12.3 | 6.1 ± 3.5 | 64.3 ± 15.6 |
| 2 | 32.6 ± 9.9 | 13.0 ± 7.2* | 43.76 ± 6.5X |
| 3 | 22.4 ± 5.7 | 5.2 ± 0.5 | 64.7 ± 1.0 |

P VS. GROUP 1
* P < 0.02
X P = 0.01

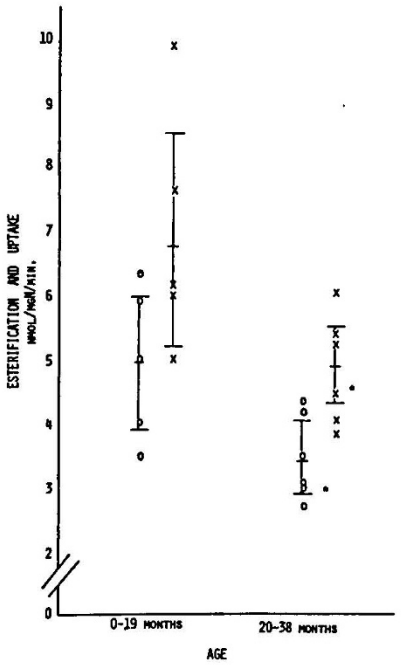


Figure Individual and mean (± 1 SD) mucosal uptake (x) and esterification (o) rates in children age 0-19 months and 20-38 months. *p = NS vs. children age 0-19 months.

thesized to triglycerides by the "triglyceride synthetase" complex enzymes before secretion into the extracellular space as chylomicrons (9,10,12,16).

The fatty acid uptake and esterification rates determined in the 14 Group 1 patients probably approximate normal values. Although the patients were referred with complaints of chronic diarrhea, sometimes with a request to exclude celiac disease, they exhibited no detectable abnormalities of gastrointestinal function other than loose stools in some cases. All patients were growing normally. The esterification rates are roughly similar to those reported in adult intestinal mucosal homogenate (2,3).

Holtzapple and associates (7) have shown that fatty acid uptake and esterification rates are inversely related to age in suckling to adult age rats. Our limited data suggest that fatty acid uptake and esterification differ little in the age groups 0-19 months and 20-38 months, respectively (see Figure). No explanation for this contradiction can be suggested. Larger numbers, including older children, are needed to verify our observations.

All children in Group 2 had intestinal mucosa epithelial injury. The group is small and not homogeneous. Fatty acid uptake and esterification were both significantly decreased and may have contributed to the steatorrhea and growth failure seen in this group. Reduction in uptake, and therefore, esterification, may be secondary to epithelial injury, but a disproportionate decrease in esterification compared to uptake is present as suggested by the reduced esterification-to-uptake ratio. The significantly increased proportion of mucosal glyceride as diglyceride with a reduction in triglyceride further suggests a specific deficiency of only one of the "triglyceride synthetase" complex enzymes, acyl CoA:diglyceride acyltransferase. This finding confirms previous work in adults with mucosal injury including celiac disease, Whipple's Disease, and "idiopathic sprue", in which reductions in esterification, independent of uptake, were present in mucosal homogenates (2,3). While an uncoupling of mucosal fatty acid uptake-esterification, with a specific reduction in acyl CoA:diglyceride transferase, is suggested by our studies in epithelial injury patients, further work in larger numbers of children is necessary to verify these findings.

Three patients with isotopic fecal excretion (1) or bile acid kinetic proven bile acid malabsorption comprised Group 3 (5). Significant reductions in esterification ($p < 0.05$) with normal uptake in the absence of mucosal damage suggest that chronic substrate limitation in humans may inhibit esterification. Previous rat studies have shown similar findings. In biliary fistula rats, triglyceride synthetase activity falls but when bile salts are perfused with normalization of intraluminal bile salt concentrations, the esterification rate returns to control levels (11).

Minimal discomfort, low risk of complication and no radioisotope exposure make the use of mucosal biopsy a safe method for the study of biochemical lesions of the small intestinal mucosa in infants and children. By applying the principles of mucosal incubation to other areas of mucosal metabolism, new previously undefined defects associated with diarrhea states in infants and children may be explored, particularly if newer, powerful microchemical methods are exploited fully.

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19. We wish to thank the staff of the General Clinical Research Center for technical assistance, and Andrea Smith and Jessica Wise for manuscript preparation.



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21. Received for publication August 28, 1978.
22. Accepted for publication March 2, 1979.




0031-3998/79/0781-0782$02.00/0