

Uptake of Fatty Acids by Jejunal Mucosal Cells Is Mediated by a Fatty Acid Binding Membrane Protein

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Abstract

The previous identification of a membrane fatty acid binding protein (MFABP) in brush border plasma membranes of the jejunum suggested that mucosal cell uptake of fatty acids might represent a carrier-mediated transport system. For evaluation of this hypothesis cellular influx kinetics (V_0) of [^3H]oleate were examined in isolated rat jejunal mucosal cells. With increasing unbound oleate concentration in the medium V_0 was saturable ($K_m = 93 \text{ nM}$; $V_{max} = 2.1 \text{ nmol} \times \text{min}^{-1}$ per 10^6 cells) and temperature dependent with an optimum at 37°C . Pretreatment of the cells with a monospecific antibody to MFABP significantly inhibited V_0 of oleate, other long-chain fatty acids, and D-monopalmitin, but not of L-alanine. Moreover, in the *in vivo* system of isolated perfused jejunal segments the physiologic significance of MFABP in the directed overall intestinal absorption process of fatty acids was documented. In the presence of the anti-MFABP oleate absorption was markedly reduced, whereas uptake of L-alanine remained unaltered. By antibody inhibition studies it was suggested that this membrane carrier also reveals transport competence for various other long-chain fatty acids, D-monopalmitin, L-lysophosphatidylcholine, and cholesterol. These data support the hypothesis that absorption of fatty acids is mediated by a fatty acid binding membrane protein.

Introduction

Although fatty acids represent the most important source of energy for the body, the mechanisms by which they are absorbed by the gut are incompletely understood. Due to the lipophilic character of fatty acids it was long assumed that they might diffuse directly through the phospholipid bilayer of the plasma membrane of mucosal cells before they undergo further intracellular metabolism (1–3). This concept was challenged by the identification of high affinity binding sites for long-chain fatty acids on microvillous membranes of the jejunum and the isolation of a 40-kD fatty acid binding protein from these membranes (MFABP)¹ (4). Therefore, it was sug-

gested that the translocation of fatty acids across the brush border membrane of jejunal mucosal cells might be mediated by such a carrier protein representing a site of metabolic and hormonal control of fatty acid absorption. For further evaluation of this hypothesis, influx kinetics of a representative long-chain fatty acid, [^3H]oleate, were examined in isolated rat jejunal mucosal cells as well as [^3H]oleate absorption by the *in vivo* system of continuously single pass perfused jejunal segments.

Methods

Materials. [$^9,10\text{-}^3\text{H}$]Oleic acid; [$1\text{-}^{14}\text{C}$]arachidonic acid; [$9,10\text{-}^3\text{H}$]palmitic acid; [$1\text{-}^{14}\text{C}$]linoleic acid; L-[$2,3\text{-}^3\text{H}$]alanine; [$1,2\text{-}^{14}\text{C}$] and [$1,2\text{-}^3\text{H}$]polyethylene glycol 4000 ([^{14}C]PEG; [^3H]PEG) and Aquasol were from New England Nuclear, Dreieich, FRG. L-Lysophosphatidylcholine-1-[^{14}C]palmitoyl and [$1\alpha,2\alpha\text{-}^3\text{H}$]cholesterol were from Amersham Corp., Braunschweig, FRG. Collagenase (type I) was from Worthington Biochemical Co., Freehold, NJ. BSA (fraction V, essentially fatty acid free), D-glucose, L-glutamine, L-alanine, insulin (bovine), polyethylene glycol 4000, ouabain, oleic acid, arachidonic acid, linoleic acid, palmitic acid, palmitoleic acid, petroselinic acid, myristic acid, stearic acid, lauric acid, capric acid, caprylic acid, caproic acid, methyl stearate, L-lysophosphatidylcholine-1-palmitoyl, cholesterol, Hepes, and phospholipase C (type XII) were from Sigma Chemie GmbH, Deisenhofen, FRG.

Preparation of D-1-monopalmitin. To a solution of $6 \mu\text{mol}$ L-lysophosphatidylcholine-1-palmitoyl in 1.5 ml of ethyl ether/95% ethanol (98:2; vol/vol) $45 \mu\text{l}$ of a 0.02-M CaCl_2 solution and $60 \mu\text{l}$ of a solution of phospholipase C (1 mg/ml) were added and incubated for 2 h at 25°C (5). Then the solvent was evaporated; the residue was dissolved in 10 ml methanol/chloroform (1:1, vol/vol), and 4.5 ml H_2O was added. After mixing, the phases were separated by centrifugation, and the chloroform phase was concentrated by evaporation under a N_2 gas stream. For separation of the monoglycerides a preparative TLC technique was employed using isopropyl ether/acetic acid (96:4, vol/vol) as first solvent and then petroleum ether/ethyl ether/acetic acid (90:10:1; vol/vol/vol) in the same direction (6). D-1-[^{14}C]Monopalmitin was prepared by the same technique adding tracer amounts of L-lysophosphatidylcholine-1-[^{14}C]palmitoyl to $6 \mu\text{mol}$ of cold L-lysophosphatidylcholine-1-palmitoyl.

All reagents were of analytical grade and doubly distilled, deionized water was used in all experiments. Glassware was acid washed.

Wistar rats were fed a standard Altromin 1314 diet and were obtained from the Zentralinstitut für Versuchstiere, Hannover, FRG.

Uptake studies of [^3H]oleate with isolated jejunal mucosal cells

Preparation of mucosal cells. Jejunal mucosal cells of overnight fasted male Wistar rats ($200\text{--}250 \text{ g}$ body wt) were prepared by a vascular collagenase perfusion technique (7). After isolation, the mucosal cells were filtered through nylon gauze ($70 \mu\text{m}$ pore size), centrifuged at 70 g for 2 min and washed twice by addition of incubation medium containing 125 mM NaCl , 2.6 mM KCl , 5.7 mM Na_2HPO_4 , 1.2 mM KH_2PO_4 , 10 mM Hepes, and 5.5 mM glucose (pH 7.4). Thereafter, the cells were diluted to 2×10^6 cells/ml in incubation medium (20°C). To maintain the physiological transcellular ion gradients of Na^+ and K^+ , it

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1. Abbreviations used in this paper: MFABP, membrane fatty acid binding protein; MVM, microvillous plasma membranes.

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was essential to use the cells within 2 h after preparation and to prevent their exposure to a temperature below 20°C, which markedly reduces Na⁺/K⁺-ATPase (8).

Viability and purity of the mucosal cell preparations were assessed by phase contrast microscopy and trypan blue exclusion capacity. Additional criteria of viability were based on the intracellular K⁺-concentration determined by atomic absorption spectroscopy as well as the release of lactate dehydrogenase (LDH) (9).

Cellular uptake of [³H]oleate. Working solutions with [³H]oleate/albumin complexes were prepared in incubation medium as previously described (4). In such solutions the concentration of unbound fatty acids was calculated by the stepwise equilibrium constant method of Wosilait and Nagy (10), employing the dissociation constant for the oleate/albumin complex reported by Spector et al. (11). The [³H]oleate working solutions were incubated with 125 μl of the isolated mucosal cells (2 × 10⁶ cells/ml) in polypropylene tubes at a final volume of 1 ml (shaking waterbath, 37°C). After certain incubation periods 200-μl sample aliquots were pipetted into 3 ml of ice-cold 0.5% albumin in incubation medium to stop cellular influx and efflux, and to remove surface bound fatty acids. After gentle mixing for 2 min on ice, the samples were pipetted onto the center of a Whatman GF/C filter (24 mm) at a rate equal to the rate of filtration under 50 mmHg vacuum pressure using a filtration apparatus (model 7 H; Hoefer Scientific, San Francisco, CA). The cells were washed with 5 ml of the 0.5% albumin solution (4°C) and thereafter with 20 ml incubation medium. Washing with more of the stop solution did not change the radioactivity remaining on the filter. The filters were placed in scintillation vials, 10 ml of Aquasol were added and the radioactivity was determined in a 1217 Rackbeta liquid scintillation counter (LKB-Wallac, Turku, Finland). Nonspecific association of radioactivity to filters and cells was determined in each experiment by adding the cold stop solution before the addition of corresponding aliquots of cells and [³H]oleate working solutions. This blank always constituted < 2% of the incubated radioactivity and was subtracted from values measured in the corresponding cellular uptake experiment. All incubations were performed in triplicate and all observations were confirmed with at least three separate cell preparations. The fraction of [³H]oleate that was esterified as well as the amount oxidized to CO₂ during the course of uptake was determined as described (12).

Further characterization of [³H]oleate transport. In studies in which the effect of Na⁺-depletion on uptake was examined, 125 mM NaCl in the incubation medium was isoosmotically replaced by sucrose, choline chloride, LiCl, or KCl. First, isolated mucosal cells were washed three times with the corresponding Na⁺-free incubation medium. Thereafter, the cells were incubated for 20 min at 37°C in the same Na⁺-free buffer, before 173 μM [³H]oleate/albumin (1:1) was added and the initial rate of uptake was determined as described. The effect of ouabain on cellular [³H]oleate influx was tested by incubation of 125 μl of the isolated mucosal cell suspension (2 × 10⁶ cells/ml) with 125 μl 4 mM ouabain in the incubation medium (37°C). Controls were pretreated with 125 μl of incubation medium alone instead of ouabain. Thereafter, the cells were incubated with 173 μM [³H]oleate/albumin (1:1) as described above. After each preincubation procedure in medium with or without Na⁺ and in presence or absence of ouabain, viability of the cells was newly determined.

Antibody inhibition studies. Outbred New Zealand white rabbits were immunized with the rat liver MFABP as reported (13). Presence and purity of the raised antibodies was examined by radial double immunodiffusion on agar plates (14) as well as by immunoblot techniques (15) with MFABP isolated from rat liver sinusoidal plasma membranes and from jejunal microvillous plasma membranes (MVM), Triton X-100 solubilized rat jejunal MVM, concentrated rat mucosal cytosolic proteins, serum albumin, and whole rat serum. The antibody to the liver MFABP cross-reacted with MFABP isolated from jejunal MVM and revealed only a single precipitin line with the total mixture of solubilized MVM proteins at 40 kD. In contrast, no reactivity was observed against cytosolic or serum proteins. For antibody inhibition studies, first the IgG fraction of this antiserum was prepared

by standard techniques (16). Then it was shown that this monospecific antibody selectively inhibited binding of [³H]oleate to isolated jejunal MVM as reported earlier (4). For determination of the effect of this antibody to MFABP on cellular influx of fatty acids, 2 ml of the cell suspension (2 × 10⁶ cells/ml) was incubated for 30 min at room temperature in gently rotating polypropylene tubes with 20–400 μg of the IgG fraction of the antiserum to MFABP or of the preimmune serum as controls. After centrifugation and washing three times in medium, the viability of the cells remained > 90% as determined by trypan blue exclusion. They were then diluted to 2 × 10⁶ cells/ml and uptake of [³H]oleate was examined as described above. Similarly, uptake of other long-chain fatty acids and monopalmitin (173 μM [¹⁴C]linoleate, [¹⁴C]arachidonate, [³H]palmitate, and D-[¹⁴C]monopalmitin in presence of albumin at a molar ratio of 2:1) was determined in mucosal cells pretreated with the IgG fraction of the anti-MFABP serum or as controls with the IgG fraction of the preimmune serum. For comparison to the fatty acid transport system, the uptake kinetics of L-[³H]-alanine were analyzed. Under identical conditions as described for [³H]oleate, 0.2–10 mM L-[³H]alanine were incubated with isolated mucosal cells. To stop cellular influx after certain incubation periods, 200-μl sample aliquots of the incubation system were pipetted into 3 ml of the medium (4°C), filtered, and further processed as described. In preceding experiments it was shown that uptake of all L-[³H]alanine concentrations employed was maximal and linear over the initial 20-s incubation period, representing cellular influx, while it gradually decreased thereafter as a result of beginning cellular efflux. Therefore, the initial rate of uptake was determined from the difference between L-[³H]alanine taken up at 5 and 20 s. For determination of the effect of the antibody to MFABP on uptake of L-[³H]alanine, the cellular influx rate was determined in mucosal cells pretreated with the IgG fraction of the antiserum or preimmune serum as controls.

Absorption of [³H]oleate by in vivo single pass perfused jejunal segments

Perfusion system. In pentobarbital anesthetized, overnight fasted, male Wistar rats (200–250 g body wt) jejunal segments with a length of 10 cm beginning 5 cm distal to the ligament of Treitz were isolated. In control experiments colonic segments with a length of 10 cm were prepared. After careful digital expulsion of stool residues, the proximal and distal ends of both segments were cannulated. Thereafter, the segments were returned to the abdominal cavity, and the abdominal wall was closed. The loops were initially washed with saline (37°C) at a rate of 7 ml/min using a multispeed transmission peristaltic pump (Bromma Multiperplex; LKB Instruments, München, FRG) for 10 min. Then the content was blown out to remove the residual isotonic saline, before luminal perfusion was started. [³H]Oleate as well as the other labeled test substances were solubilized in 10 mM Na-taurocholate in 85 mM NaH₂PO₄/45 mM Na₂HPO₄ (pH 6.5). Perfusion media containing 1 mM methyl stearate or 1 mM cholesterol were fine emulsions, whereas all other media with lipophilic substrates were micellar solutions. Addition of 5 g/liter polyethylene glycol (PEG 4000) as a nonabsorbable marker (containing [¹⁴C]PEG; 5 μCi/liter) served to determine possible fluid shifts during the experimental procedure. Only in experiments where uptake of ¹⁴C-labeled compounds was examined was PEG added as [³H]PEG. The media used for the perfusion experiments were mixed with a high-speed ultrasonic homogenizer and during perfusion by a magnetic stirrer. The temperature of the continuously stirred infusate was kept at 37°C. The standard rate of perfusion was 0.4 ml/min for 30 min with 10-min collection periods. After each experiment the perfused segments were removed and their lengths and weights were determined. The length of jejunal segments was 10.4 ± 0.9 cm, weighing 9.8 ± 1.1 g, whereas colonic segments (9.8 ± 0.9 cm) weighed 16.1 ± 1.7 g. In representative experiments the composition of the [³H]oleate solution in the effluent was examined (12, 17). For the various concentrations employed it was demonstrated that the effluent recovered [³H]oleate was still in the unesterified form.

Moreover, it was analyzed whether the specific activity of [³H]-

oleate in the infusate and effusate was changed, e.g., due to the admixture of fatty acids generated by hydrolysis of lipids in shed epithelial cells. For this purpose the free fatty acid fractions of defined volumes of the infusate (1 mM [³H]oleate) and the effusate were prepared by thin-layer chromatography (12, 17). The fatty acid composition of these preparations was quantitatively determined by a gas-liquid chromatography technique using a C₁₇ fatty acid as internal standard as described earlier (17). It was shown that only C_{18:1} fatty acids were present in the infusate and effusate samples. The quantity of these fatty acids was correlated to the radioactivity in defined aliquots of both media. Since the radioactivity per mass of fatty acid was identical in the infusate and in the effluent, it was concluded that the specific activity did not change during the course of the experiment.

Radioactivity determinations. 100- μ l aliquots of the effluent fractions were withdrawn in triplicates from each 10-min collection period, 10 ml of Aquasol was added, and the radioactivity was measured for determination of the nonabsorbed [³H]oleate or other labeled compounds. Similarly, infusate samples were taken to analyze the specific activity of the applied substrate. Quench correction was made by external standardization. Samples containing two isotopes were counted in two channels. Counts per minute were converted to disintegrations per minute for each isotope with a computer program, which corrected for quenching and spillover of ¹⁴C into the ³H channel (18). Spillover of ³H into the ¹⁴C channel was < 1%.

Absorption calculations. For all perfusion studies, it was shown that the recovery of PEG in the effluent was between 98 and 100%, indicating that no significant net fluid shifts took place under the experimental conditions employed (see Results). Since the specific activity of [³H]oleate also did not change during the course of the experiments, the absorption of [³H]oleate was determined from the difference between infused and effluent recovered radioactivity. The absorption rates were calculated according to the equation (19): $R = \text{DPM}_0 - \text{DPM}_t / \text{DPM}_0 \times A \times V / T \times L$, where R is the absorption rate (nmols \times min⁻¹ \times cm⁻¹); DPM_0 and DPM_t are measured, quench-corrected activities of [³H]oleate at zero time and at a given time, respectively. A is the amount (nanomoles) of oleate in 1 ml of perfusate; V is the volume (milliliters) of perfusate within a given interval; T is the time period (minutes) of sample collection; and L is the length (centimeters) of the perfused segment.

Absorption studies included the following experiments. (A) The concentration dependency of the overall mucosal fatty acid uptake was analyzed by perfusion with clear micellar solutions containing 0.05–3 mM [³H]oleate. (B) For examination of the effect of Na⁺ on fatty acid absorption, uptake of [³H]oleate in presence of Na⁺ was compared to uptake in absence of Na⁺ by isoosmotic replacement of Na⁺ by K⁺ in the perfusion buffer. (C) It was examined, whether presence of other substrates in the perfusion medium had any effect on [³H]oleate absorption. Therefore, equimolar concentrations of various short-, medium-, and long-chain fatty acids, methyl stearate, D-monopalmitin, L-lysophosphatidylcholine, cholesterol, D-glucose, and L-alanine were added separately to the 1 mM [³H]oleate containing perfusion medium. Each experiment was performed in three different rats ($n = 3$).

Determination of the monomeric activity of oleic acid in the medium. As an estimation of the monomeric fatty acid concentration in media with and without Na⁺ the uptake of protonated [³H]oleic acid by polyethylene disks was determined according to Sallee (20). Polyethylene disks (1 cm²) were cut from polyethylene film, 1 mm thick. To remove any oil and debris the disks were washed in methanol and distilled water, and were dried before use. After equilibration in 5 ml of the [³H]oleate test solutions for 24 h (shaking waterbath, 37°C), the disks were removed, rinsed vigorously in buffer, and the radioactivity associated with the disks was determined as described above.

Antibody inhibition studies. To determine the effect of the antibody to MFABP on the overall fatty acid absorption process, the lumen of the jejunal segment was perfused for 30 min (0.4 ml/min; recirculating system) with PBS containing per 100 ml 10 mg of the IgG-fraction of the antiserum or in control experiments 10 mg of the IgG-fraction of the preimmune serum. Thereafter, recirculation was discontinued and

the lumen single pass perfused for 15 min (equilibration period) with IgG-free medium, before absorption of [³H]oleate and other test substances was examined as described above. For determination of the viability of the jejunal mucosa after this pretreatment procedure, uptake of L-[³H]alanine was examined.

Statistical analysis. Results are given as means \pm SD. The t test was used to test for significant differences among means (21). The kinetics of [³H]oleate uptake rates as a function of the oleate concentration in the medium were determined by fitting the weighted uptake data by computerized least square regression as described (22). Derived kinetic parameters (K_m and V_{max}) in presence and absence of the antibody to MFABP were tested for significant differences with a z -test (21). P values ≤ 0.05 were considered significant.

Results

Uptake of [³H]oleate by isolated jejunal mucosal cells

Validation of methods. Phase contrast microscopy of the isolated mucosal cell preparations demonstrated that 91 \pm 3% of the cells revealed the typical features of intestinal epithelium with oval or elongated cell bodies, basal nuclei, and prominent brush borders at the apical pole. 94 \pm 4% of the cells excluded trypan blue; the intracellular K⁺-concentration remained > 85 mM; and loss of cellular LDH was < 15% during 3 h after preparation of the cells. For determination of the functional integrity of the cells their uptake competence for L-[³H]alanine, which is known to be actively transported, was examined. With increasing L-[³H]alanine concentrations incubated, its cellular influx velocity revealed saturation kinetics (criterion of carrier-mediated transport) with a K_m of 2.3 \pm 0.4 mM and a V_{max} of 17.0 \pm 2.4 nmol \times min⁻¹ per 10⁶ mucosal cells.

To determine the amount of [³H]oleate taken up by the cells after certain times of incubation without the fraction of ligand merely bound to the outer surface of the plasma membrane, it is necessary to use a stop/chase solution, which immediately stops the membrane translocation process and removes nontransported ligand from the plasma membrane. In previous experiments it was shown that the use of a 0.5% albumin solution at 4°C represents such an effective stop/chase solution (23). Therefore, in experiments with isolated mucosal cells it was evaluated whether the addition of 3 ml of this ice-cold 0.5% albumin solution to 200- μ l aliquots of the incubation system is effective in stopping cellular influx and efflux, and in removing of surface bound fatty acids. Aliquots of 25 μ l of the mucosal cell suspension (2 \times 10⁶ cells/ml) were incubated with 173 μ M [³H]oleate/albumin (1:1) in 200 μ l of the incubation medium for 30 s. Immediately thereafter (zero time) 3 ml of ice-cold 0.5% albumin in incubation buffer was added and radioactivity remaining with the cells was determined at intervals up to 15 min. During a rapid reequilibration period of maximal 1 min 43 \pm 5% of the cell associated radioactivity observed immediately after the 30-s incubation period was removed. However, over the next 15 min no further loss of cellular [³H]oleate was detectable. Increasing the albumin concentration in the stop/chase solution up to 3% did not significantly decrease the amount of cell associated radioactivity, indicating that removal of surface bound oleate is already complete at albumin concentrations of 0.5% and leakage of internalized fatty acids is not inducible by higher albumin concentrations. The suggestion that the initial loss of radioactivity observed immediately after the 30-s incubation period represents [³H]oleate effectively chased off membrane

binding sites was further supported by the following observation: Incubation of mucosal cells with [^3H]oleate at 4°C for 30 s, a condition where no transport but membrane binding of ligands is to be expected, resulted in only 47±6% of the values of cell associated radioactivity observed at 37°C. Subsequent addition of 0.5% albumin (4°C) led to a > 90% loss of this membrane bound [^3H]oleate.

Uptake kinetics of [^3H]oleate by isolated mucosal cells. Uptake of [^3H]oleate was examined at various unbound oleate concentrations in the medium. The unbound oleate concentration was modulated without exceeding its solubility (24, 25) by incubation of a fixed concentration of 173 μM [^3H]oleate bound to varying concentrations of albumin, or by incubation of a fixed concentration of albumin and increasing concentrations of [^3H]oleate, such that the oleate/albumin molar ratio varied between 0.2:1 up to 2:1. This provided a range of calculated unbound oleate concentrations of 17–401 nM. For all oleate-albumin complexes the initial cellular uptake velocity was determined as a measure of the actual translocation process of fatty acids across the plasma membranes. These unidirectional cellular influx rates were defined as the maximal and linear initial phases of the cumulative uptake curves. Accordingly, the time course of [^3H]oleate uptake at all oleate/albumin molar ratios incubated was examined. For each oleate-albumin complex it was shown that over the initial 30-s incubation period uptake was maximal and linear, whereas it gradually decreased thereafter as a result of beginning cellular efflux (Fig. 1; illustrated is a representative experiment with 173 μM [^3H]oleate/albumin [1:1]). The initial uptake phase was independent of cellular fatty acid metabolism, since after 30 s 85±11% of the intracellular fatty acids were still in the unesterified form and fatty acid oxidation was not detectable. For evaluation whether the initial uptake component reveals criteria of a carrier-mediated uptake process, this cellular influx rate was determined as a function of the calculated unbound oleate concentration in the medium. As shown in Fig. 2, with increasing unbound oleate concentration incubated uptake followed saturation kinetics with a K_m of 93±8.9 nM and a V_{\max} of 2.10±0.19 nmol \times min $^{-1}$ per 10 6 mucosal cells. Since maximal uptake over the initial 30-s incubation period never exceeded 2% of the incubated substrate, and the unbound monomeric oleate concentration in the medium was

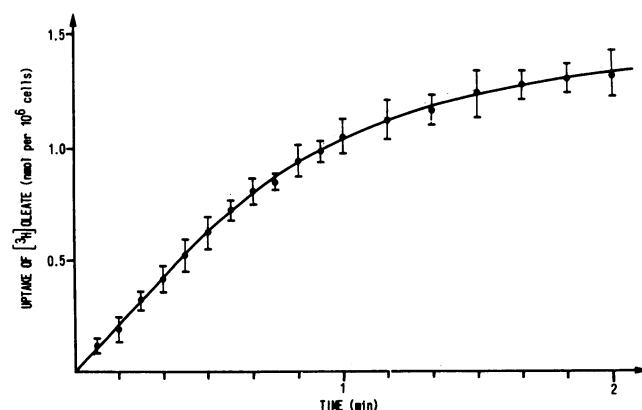


Figure 1. Time course of [^3H]oleate uptake. 173 μM [^3H]oleate/albumin (1:1) were incubated in 1 ml incubation medium at 37°C with 125 μl of isolated jejunal mucosal cells (2×10^6 cells/ml). Values are means±SD of three replicate experiments.

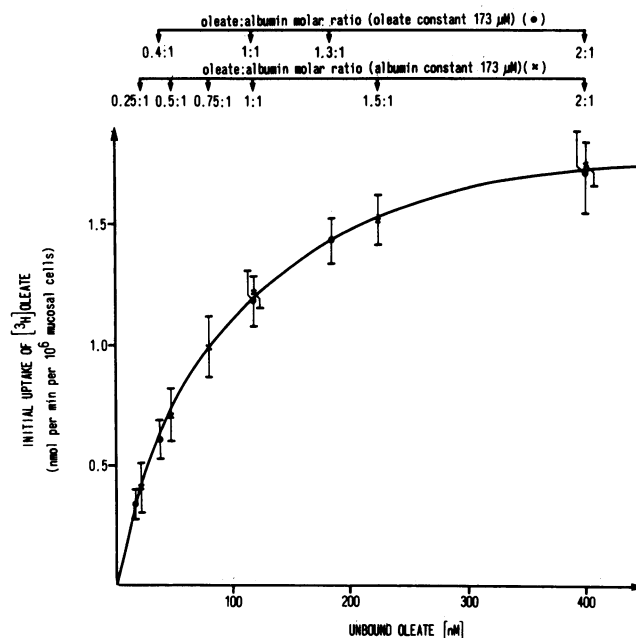


Figure 2. Initial uptake rate as a function of the unbound oleate concentration in the medium. Isolated mucosal cells (0.25×10^6 cells) were incubated in 1 ml medium at 37°C with a fixed concentration of 173 μM [^3H]oleate bound to various concentrations of albumin or with various concentrations of [^3H]oleate (43–346 μM) bound to a fixed concentration of 173 μM albumin. The unbound oleate concentration was calculated as described in Methods. The initial uptake rates were determined from the linear slopes of the cumulative uptake curves over the initial 30-s incubation period. Values are means±SD of three replicate experiments. The kinetic parameters were generated from a weighted least-squares fit of the individual data points from each experiment to a rectangular hyperbola (K_m 93.1±8.9 nM; V_{\max} 2.10±0.19 nmol \times min $^{-1}$ per 10 6 mucosal cells).

well below the critical concentration for the formation of micelles (24, 25), it is evident that the apparent saturation kinetics were real and did not reflect substrate depletion. Uptake was also not limited by the oleate-albumin dissociation rates, since the maximal uptake velocity was at least 100-fold slower than the rate of spontaneous dissociation of all oleate-albumin complexes incubated (26). Overlap of the [^3H]oleate uptake curves, obtained by varying either the oleate or albumin concentration in the incubation system, demonstrates that diffusional gradients due to the differing concentrations of albumin are of no significance under the experimental conditions employed. This observation of saturable fatty acid uptake kinetics was considered to be a criterion of a carrier-mediated transport mechanism.

Characterization of the [^3H]oleate cellular uptake process. To evaluate the effect of Na^+ on fatty acid uptake, cellular influx of 173 μM [^3H]oleate/albumin (1:1) was examined in presence of Na^+ as well as in its absence, by isoosmotic replacement of NaCl by sucrose, LiCl , KCl , or choline chloride in the incubation medium. Irrespective of the nature of the various substitutes, in absence of Na^+ fatty acid influx was reduced by 51–61% compared to the presence of Na^+ (Table I). This inhibition of influx was not due to a decrease of the monomeric activity of [^3H]oleate in the incubation medium, which remained unaltered as determined by the polyethylene disk method (7.2±0.4 nmol per disk in presence of Na^+ ; 7.8±0.5

Table I. Effect of Na⁺ Substitution on Cellular Influx of [³H]Oleate

| Medium with | Influx rate |
|-------------|---|
| | <i>nmol × min⁻¹ per 10⁶ cells</i> |
| NaCl | 1.09±0.19 |
| LiCl | 0.45±0.05* |
| KCl | 0.42±0.10* |
| Choline Cl | 0.48±0.07* |
| Sucrose | 0.53±0.11* |

125 mM NaCl in the incubation medium were isoosmotically replaced by sucrose, choline Cl, LiCl, or KCl. Values are means±SD of three replicate experiments. For statistical analysis influx rates in absence of Na⁺ were compared to controls in presence of Na⁺; differences (*P* < 0.05) were marked by an asterisk.

nmol per disk in absence of Na⁺). Trypan blue exclusion studies revealed that the viability of the cells did not significantly drop in absence of Na⁺ (92±2%) compared to the presence of Na⁺ (96±4%; *P* > 0.05).

To determine whether fatty acid influx is linked to the membrane Na⁺/K⁺-ATPase, 173 μM [³H]oleate/albumin (1:1) was incubated with mucosal cells pretreated with 2 mM ouabain, a known inhibitor of this enzyme. Uptake in ouabain-pretreated cells revealed a 41% inhibition of the initial rate of uptake (0.69±0.09 vs. 1.17±0.12 nmol × min⁻¹ per 10⁶ cells in controls not pretreated with ouabain, *P* < 0.001; viability of both cell preparations > 90%).

Evaluation of MFABP as carrier protein. The observation of a saturable uptake component is compatible with the hypothesis of a facilitated diffusion membrane transport process. In fact, as putative carrier protein a 40-kD fatty acid binding membrane protein (MFABP) was previously isolated from microvillous membranes of the jejunum (4). With an antibody to MFABP, binding of fatty acids to microvillous membranes of the jejunum was selectively inhibited (4). For evaluation whether MFABP may also mediate fatty acid influx into isolated jejunal mucosal cells, the effect of this monospecific antibody on the initial rate of cellular [³H]oleate uptake was determined. When [³H]oleate uptake by mucosal cells pretreated with the IgG-fraction of the antiserum to MFABP was compared to cells pretreated with the IgG-fraction of the preimmune serum, a significant inhibition of initial uptake velocity by the antibody was demonstrated. This inhibition was dependent on the concentration of the antibody, reaching a maximum at 100 μg IgG per incubation. Studies on influx kinetics as a function of the incubated unbound oleate concentration revealed a predominant noncompetitive type of inhibition of uptake by the anti-MFABP (Fig. 3). This was evident by a marked decrease of *V*_{max} (0.651±0.087 nmol × min⁻¹ per 10⁶ cells in antibody pretreated cells vs. 1.917±0.213 nmol × min⁻¹ per 10⁶ cells in controls; *P* < 0.001), but no statistical difference in the obtained *K*_m values (94.0±8.1 vs. 89.3±9.4 nM in controls; *P* > 0.05).

Furthermore, the effect of this antibody to MFABP on cellular influx of other long-chain fatty acids was evaluated. 173 μM [¹⁴C]linoleate, [¹⁴C]arachidonate, [³H]palmitate, and D-[¹⁴C]monopalmitin, all in presence of albumin at a molar ratio of 2:1, were used for these incubation studies. In antibody pretreated mucosal cells the cellular influx rate of these fatty

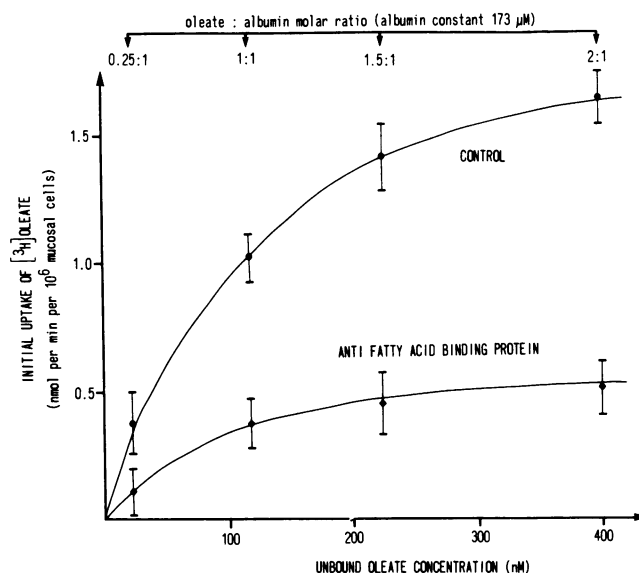


Figure 3. Inhibition of [³H]oleate influx into isolated mucosal cells by the anti-MFABP. Cells pretreated with 100 μg of the IgG fraction of the antiserum were compared to control preparations pretreated with 100 μg of the IgG fraction of the preimmune serum. 125 μl of such cell suspensions (2 × 10⁶ cells/ml) were incubated with increasing concentrations of [³H]oleate (43–346 μM) bound to a fixed concentration of 173 μM albumin at 37°C. Illustrated are the initial uptake rates as a function of the calculated unbound oleate concentrations in the incubation medium. Values are means±SD of three replicate experiments.

acids and D-monopalmitin was inhibited by 66–73% compared with control cells treated with the IgG-fraction of the preimmune serum (Table II).

To determine whether the inhibition of fatty acid uptake by the antibody marks a specific effect on the cellular fatty acid uptake system or represents a more general interference with membrane transport processes, the effect of this antibody on cellular influx of another actively transported substrate, L-[³H]alanine, was examined. Uptake kinetics of L-[³H]alanine by mucosal cells pretreated with the IgG fraction of the antiserum (*K*_m = 2.11±0.18 mM; *V*_{max} = 19.17±2.5 nmol × min⁻¹

Table II. Effect of the Antibody to MFABP on Cellular Influx of Various Long-Chain Fatty Acids and Monopalmitin

| Incubated substrate | Cellular influx | | Inhibition % |
|---------------------|---|-------------|--------------|
| | Anti-MFABP | Controls | |
| | <i>nmol × min⁻¹ per 10⁶ cells</i> | | |
| Palmitate | 0.508±0.071 | 1.509±0.174 | 66 |
| Oleate | 0.474±0.097 | 1.732±0.207 | 73 |
| Linoleate | 0.512±0.112 | 1.851±0.181 | 72 |
| Arachidonate | 0.527±0.081 | 1.897±0.163 | 72 |
| Monopalmitin | 0.429±0.075 | 1.496±0.121 | 71 |

Mucosal cells pretreated with the IgG fraction of the antiserum to MFABP were compared to cells pretreated with the IgG fraction of the preimmune serum (controls). The radiolabeled substrates were incubated at concentrations of 173 μM in presence of 86.5 μM albumin. Values are means±SD.

per 10^6 mucosal cells) did not significantly differ from those pretreated with the IgG-fraction of the preimmune serum ($K_m = 2.47 \pm 0.26$ mM; $V_{max} = 17.7 \pm 2.0$ nmol \times min $^{-1}$ per 10^6 mucosal cells; $P > 0.05$).

Absorption of [3 H]oleate by isolated perfused jejunal segments

Analysis of the overall fatty acid absorption process. Absorption of fatty acids was analyzed in the in vivo system of continuously single-pass perfused jejunal segments. First it was determined whether under the experimental conditions employed fluid shifts may take place and therefore may alter the oleate uptake data. By analysis of the concentration of nonabsorbable PEG in the infusate as well as in the effluent, < 1% net fluid absorption was detectable. Therefore, the experimental results were not corrected for fluid shifts. During the perfusion experiments steady state conditions were reached after 10 min, since there was no difference in the absorption rates between the 10- to 20-min and 20- to 30-min collections. The concentration dependency of [3 H]oleate absorption rates was studied in micellar solutions containing 0.05–3 mM [3 H]oleate

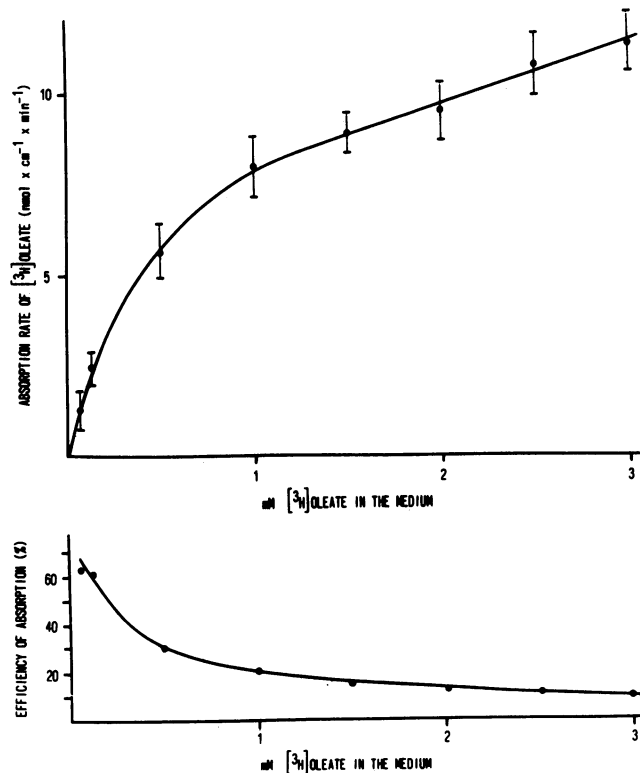


Figure 4. Absorption rate of [3 H]oleate as a function of its concentration in the perfusion medium. Isolated jejunal segments were in vivo perfused with 0.05–3 mM [3 H]oleate in presence of 10 mM Na $^+$ -taurocholate at 0.4 ml \times min $^{-1}$. Results are given as means \pm SD of three replicate experiments. The best fit curve of the total absorption rates obtained by computerized least square regression of the experimental data is shown. This absorption curve was resolved into two components, a nonsaturable passive absorption component and a single Michaelis-Menten component with a K_m of 0.271 mM and a V_{max} of 8.125 nmol \times cm $^{-1}$ \times min $^{-1}$, which was predominant at low substrate concentration. In the lower part of the figure the efficiency of [3 H]oleate absorption is illustrated. It was calculated for each concentration of oleate in the medium as mean percentage absorption of the amount infused per hour.

and 10 mM Na $^+$ -taurocholate (pH 6.5) (Fig. 4). With increasing concentrations of [3 H]oleate applied the absorption rates increased in a nonlinear fashion. Computerized fitting of the uptake values revealed a dual concentration dependent absorption mechanism. At higher intraluminal concentrations (1–3 mM) the relationship between the concentration of oleate and the absorption rate was linear with a slope of 1.706 nmol \times cm $^{-1}$ \times min $^{-1}$ per mmol/liter oleate in the medium, suggesting a passive diffusional transport mechanism. The other uptake component revealed Michaelis-Menten kinetics with a V_{max} of 8.125 nmol \times cm $^{-1}$ \times min $^{-1}$ (half saturation at 0.271 mM [3 H]oleate applied). This saturable absorption mechanism was the predominant process of transport at low intraluminal fatty acid concentrations. Accordingly, the efficiency of [3 H]oleate absorption at low concentrations (0.05–0.5 mM) was significantly higher ($P < 0.01$) compared with the uptake rates at high (> 1 mM) intraluminal fatty acid concentrations (Fig. 4).

Characterization of the overall fatty acid absorption process. To determine whether absorption of [3 H]oleate is affected by other substrates, various short-, medium-, and long-chain fatty acids, methyl stearate, D-monopalmitin, L-lysophosphatidylcholine, cholesterol, L-alanine, and D-glucose were added separately at concentrations of 1 mM to micellar solutions containing equimolar concentrations of [3 H]oleate (1 mM) and 10 mM Na $^+$ -taurocholate at pH 6.5. As shown in Table III, absorption of [3 H]oleate was neither affected by the addition of short- and medium-chain fatty acids, nor in presence of L-alanine or D-glucose. In contrast, long-chain fatty acids, fatty acid methyl ester, monoglyceride, L-lysophosphatidylcholine, and cholesterol reduced [3 H]oleate uptake significantly ($P < 0.01$).

Table III. Influence of the Addition of Various Unlabeled Substrates on 1 mM [3 H]Oleate Absorption

| Additive | Absorption of [3 H]oleate |
|---------------------------|---|
| 1 mM | nmol \times cm $^{-1}$ \times min $^{-1}$ |
| None | 7.131 \pm 0.942 |
| Caproic acid | 7.322 \pm 0.976 |
| Caprylic acid | 7.204 \pm 0.793 |
| Capric acid | 7.041 \pm 0.632 |
| Lauric acid | 6.926 \pm 1.017 |
| Myristic acid | 5.635 \pm 0.554* |
| Palmitic acid | 5.072 \pm 0.631* |
| Stearic acid | 4.743 \pm 0.463* |
| Palmitoleic acid | 4.819 \pm 0.864* |
| Petroselinic acid | 4.686 \pm 0.731* |
| Linoleic acid | 4.041 \pm 0.406* |
| Arachidonic acid | 3.898 \pm 0.473* |
| Methyl stearate | 5.117 \pm 0.748* |
| D-Monopalmitin | 4.822 \pm 0.498* |
| Cholesterol | 5.378 \pm 0.612* |
| L-Lysophosphatidylcholine | 5.486 \pm 0.731* |
| D-Glucose | 7.245 \pm 0.638 |
| L-Alanine | 7.310 \pm 0.964 |

Values are means \pm SD of three replicate experiments. For statistical analysis uptake rates in presence of 1 mM unlabeled substrates were compared to controls in their absence; differences ($P < 0.01$) were marked by an asterisk.

For determination of Na⁺-dependency of fatty acid absorption, uptake of 1 mM [³H]oleate in presence of NaH₂PO₄/Na₂HPO₄-buffer was compared to the absorption rate in absence of Na⁺ by isoosmotic replacement of Na⁺ with K⁺ (KH₂PO₄/K₂HPO₄). Despite this exchange of cations the monomeric protonated oleic acid concentration in the perfusion medium remained unaltered as determined by the polyethylene disk method (presence of Na⁺: 32.9±3.4 nmol per disk; absence of Na⁺: 31.6±2.7 nmol per disk). However, in absence of Na⁺ uptake of [³H]oleate was reduced by 41% (4.655±0.397 nmol × cm⁻¹ × min⁻¹) compared with the presence of Na⁺ (7.891±0.814 nmol × cm⁻¹ × min⁻¹).

Evaluation of MFABP as responsible membrane transport protein in the overall intestinal absorption process of fatty acids. In the system of isolated perfused jejunal segments the biologic significance of MFABP as carrier protein, mediating the uptake of luminal fatty acids into the mucosal cells was analyzed. This was of particular importance, since studies with isolated mucosal cells alone cannot differentiate whether the antibody to MFABP inhibits cellular fatty acid influx at the apical and/or basolateral pole of the mucosal cells. Although previous immunohistochemical studies showed that MFABP is predominately localized at the apical site of jejunal mucosal cells (4), kinetic evidence was still lacking that it actually mediates the translocation of fatty acids across the MVM. Luminal perfusion with the IgG fraction of the antiserum to MFABP prevents interaction of the antibody with the basolateral pole of the mucosal cell and thus may allow direct determination of the effect of the monospecific antibody to MFABP on cellular uptake of fatty acids at the site of the MVM. Therefore, jejunal segments pretreated with the IgG fraction of the antiserum to MFABP were compared to control segments pretreated with the IgG fraction of the preimmune serum. The concentration dependent absorption kinetics of [³H]oleate were markedly reduced in segments pretreated with the antibody to MFABP compared to the controls (Fig. 5). Since absorption was not completely inhibited by the antibody to MFABP, it is assumed that most of the remaining fraction is absorbed by the above described diffusional transport component. Furthermore, the effect of the antibody to MFABP on absorption of various other radiolabeled long-chain fatty acids, D-[¹⁴C]monopalmitin, L-[¹⁴C]lysophosphatidylcholine, [³H]cholesterol, and L-[³H]alanine, all at concentrations of 1 mM, was examined. As shown in Table IV, uptake of the various long-chain fatty acids, D-monopalmitin, L-lysophosphatidylcholine, and cholesterol by antibody pretreated jejunal segments was inhibited by 36–53%. This suggests that the membrane fatty acid carrier protein may also reveal transport competence for other lipolytic products. In contrast, under identical experimental conditions perfusion with L-alanine revealed no difference of the absorption rates in presence or absence of the anti-MFABP. This also indicated the viability of the antibody pretreated jejunal segments.

Since the above observations suggest that fatty acid absorption by the jejunal mucosa represents at least, in part, a carrier-mediated uptake mechanism, it was of potential interest to examine uptake of [³H]oleate by an intestinal organ that is not known to participate in the long-chain fatty acid absorption process under physiological conditions. Therefore, a 10-cm colonic segment was perfused with 0.1–3 mM [³H]oleate in presence of 10 mM Na⁺-taurocholate in Na⁺-phosphate buffer (pH 6.5). Under these conditions fluid shifts were

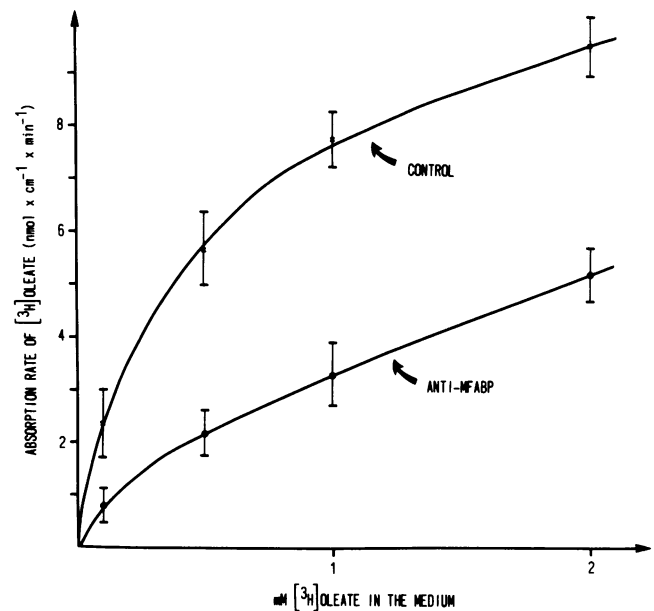


Figure 5. Effect of the antibody to MFABP on the absorption rate of [³H]oleate. Jejunal segments were pretreated with the IgG fraction of the antiserum to MFABP or of the preimmune serum as controls. Thereafter, the segments were perfused with increasing concentrations (0.1–2.0 mM) of [³H]oleate and the absorption rates were determined as described. Values are means±SD of three replicate experiments. At all [³H]oleate concentrations infused, absorption rates by jejunal segments pretreated with anti-MFABP were significantly reduced ($P < 0.001$) compared to the controls.

not detectable by PEG concentration measurements (see Methods). With increasing oleate concentrations infused the absorption rate increased as a linear function (slope: 2.131 nmol × cm⁻¹ × min⁻¹ per mmol/liter oleate infused). This corresponded to an uptake efficiency of 5.6±0.4% at all [³H]oleate concentrations applied. The slow and low efficient absorption process for long chain fatty acids in the colon was suggestive for a passive diffusional transport mechanism. This

Table IV. Effect of the Antibody to MFABP on Absorption of Various Radiolabeled Substrates

| Substrate infused | Absorption rate | | Inhibition by anti-MFABP |
|-------------------|---|-------------|--------------------------|
| | Control | anti-MFABP | |
| | nmol × cm ⁻¹ × min ⁻¹ | | % |
| Oleate | 8.107±0.931 | 3.859±0.402 | 52 |
| Arachidonate | 8.431±1.074 | 4.831±0.691 | 43 |
| Palmitate | 7.269±0.813 | 4.633±0.545 | 36 |
| Linoleate | 8.317±0.998 | 5.075±0.419 | 39 |
| D-Monopalmitin | 7.493±0.818 | 4.471±0.529 | 40 |
| L-Lyso-PC* | 7.834±0.946 | 5.012±0.368 | 36 |
| Cholesterol | 8.779±0.897 | 4.173±0.517 | 53 |
| L-Alanine | 7.198±0.960 | 7.213±0.644 | — |

Jejunal segments pretreated with the IgG fraction of the antiserum to MFABP were compared to control segments pretreated with the IgG fraction of the preimmune serum. The various substrates were then infused at a concentration of 1 mM. Values are means±SD of three replicate experiments.

* L-Lysophosphatidylcholine.

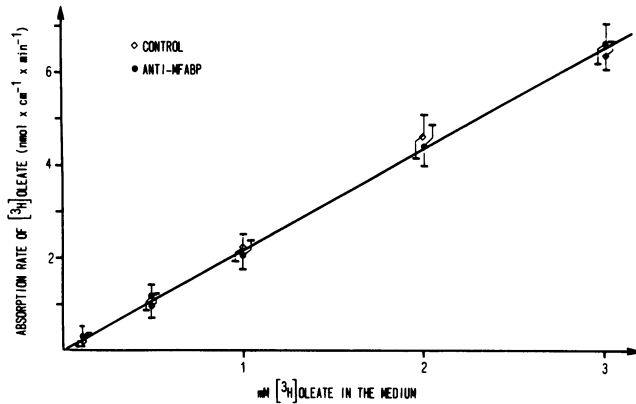


Figure 6. Effect of the antibody to MFABP on absorption rates of [³H]oleate by colonic mucosa. Colonic segments were pretreated with the IgG fraction of the antiserum to MFABP or of the preimmune serum as controls. In both preparations [³H]oleate uptake rates were not statistically different ($P > 0.05$) and increased linearly as a function of the oleate concentration in the medium. Values are means \pm SD of three replicate experiments.

hypothesis was supported by antibody inhibition studies. As shown in Fig. 6, the absorption rates of [³H]oleate were not different whether the colonic segment was pretreated with the IgG-fraction of the antiserum to MFABP or of the preimmune serum. This observation is in agreement with the immunohistochemical distribution pattern of MFABP, revealing prominent staining of the microvillous membranes of the jejunal mucosa but absent staining in the apical plasma membrane of colonic mucosal cells (4).

Discussion

The essential step in the very efficient fatty acid absorption process represents the permeation through the microvillous membrane of the jejunal mucosal cells. The identification of a fatty acid binding protein with a molecular weight of 40 kD in these membranes suggested that fatty acid entry into mucosal cells may represent a carrier-dependent transport system (4). In the present study it was demonstrated that mucosal cell uptake of fatty acids is, in fact, mediated by this fatty acid binding membrane protein. The evidence for such a carrier-mediated transport process was derived from the kinetic studies with isolated jejunal mucosal cells. In those experiments it was essential to determine the initial phases of cellular fatty acid uptake, since they represent the unidirectional translocation process across the plasma membrane, which is independent of intracellular fatty acid metabolism. Studies of this cellular influx rate as a function of the unbound fatty acid concentration in the medium revealed saturation kinetics and temperature sensitivity. The K_m value of 93 nM indicates that this transport system has a high affinity for fatty acids. This is expected since uptake occurs despite the tight binding of fatty acids to albumin in the incubated medium. In general, it is observed that cellular uptake of albumin bound lipophilic substrates reveals significant lower K_m values as hydrophilic, nonalbumin-bound compounds such as amino acids or glucose (27–29). During the absorptive phase this K_m value for cellular influx of [³H]oleate is below the estimated concentration of monomeric fatty acids in the unstirred water layer of the jejunal mucosa (30, 31). This allows fatty acid absorption

into mucosal cells to occur at maximal velocity. The observed V_{max} value of 2.1 nmol \times min per 10^6 cells for nondiffusional uptake of long-chain fatty acids distributed over the large surface area of the intestinal mucosa (32) provides a sufficient absorption capacity for fatty acids at physiological intestinal transit times. Similar K_m - and V_{max} -values observed in studies with mucosal cells, cardiomyocytes (23), and hepatocytes (33, 34) as well as identification of a MFABP with identical molecular weight in all of these three different tissues (4, 13, 23) suggested the existence of a common fatty acid uptake system. This was confirmed by immunologic studies employing a monospecific antibody to the liver fatty acid binding membrane protein. Immunoblot analysis of this antibody in dilutions of 1:1,000 to 1:50 with whole tissue homogenates of liver, heart, and jejunal mucosa revealed a single 40 kD protein in all preparations, indicating that the three MFABP share antigenic determinants. Moreover, immunofluorescence studies with this monospecific antibody showed exclusive staining of the plasma membranes of these three cell types (4, 13, 23). In sections of jejunal mucosa a predominant staining of the microvillous membrane portion of the mucosal cells was evident (4). The suggestion that this membrane protein mediates mucosal cell uptake of fatty acids was substantiated by the antibody inhibition studies presented here. It was shown that the monospecific antibody to MFABP inhibits influx of various long-chain fatty acids and the monoglyceride D-monopalmitin into isolated mucosal cells without impairing other cell functions. The predominant noncompetitive type of inhibition of [³H]oleate uptake suggested that this monospecific, polyclonal rabbit antibody may interact with various domains of the fatty acid binding membrane protein leading to conformational changes of the protein, which is accompanied by an impairment of its carrier function.

[³H]Oleate absorption by in vivo perfused isolated jejunal segments was analyzed. First it was shown that fatty acid absorption by jejunal mucosa represents a dual, concentration dependent uptake mechanism consisting of a passive diffusional transport process and an active carrier-mediated translocation mechanism, which is predominant at low substrate concentrations. In contrast, perfusion studies with colonic segments revealed only a low efficient fatty acid absorption mechanism (linear uptake kinetics) that was not inhibited by the monospecific antibody to MFABP. This was suggestive for a carrier independent, passive diffusional transport process. For further evaluation of the jejunal fatty acid absorption mechanism, the influence of various unlabeled substrates on 1 mM [³H]oleate uptake was determined. The separate additions of equimolar concentrations of various long-chain fatty acids, methyl stearate, D-monopalmitin, L-lysophosphatidylcholine, and cholesterol significantly decreased the absorption rate of [³H]oleate by up to 45% (Table III). This may in part be due to an enlargement of the micelles in the perfusate, which reduces their diffusion rate across the unstirred water layer towards the absorptive cell membrane (30, 31, 35). Alternatively, it seems possible that these compounds compete with [³H]oleate for binding sites at the MFABP, serving as common membrane fatty acid carrier. In contrast, uptake of [³H]oleate was not affected by the addition of short- and medium-chain fatty acids, D-glucose and L-alanine, suggesting that these substrates do not interfere with the micelle formation process and/or are taken up by a different transport mechanism.

To evaluate the biologic significance of MFABP as carrier

in the absorption process of fatty acids, antibody inhibition studies were performed. In contrast to isolated mucosal cells the experimental model of perfused jejunal segments provides the advantage that the physiologic polarity of the absorption process is maintained. In this system it is ensured that luminal application of the antibody to MFABP leads only to a reaction with the fatty acid carrier proteins located at the MVM of jejunal mucosal cells. It was in fact shown that pretreatment of the mucosa of isolated perfused jejunal loops with the monospecific antibody to MFABP resulted in a significant inhibition of the concentration dependent intestinal uptake of [³H]oleate (Fig. 5). This confirms the significance of MFABP as the responsible MVM carrier protein in the directed overall absorption process of fatty acids.

For determination of the specificity of this intestinal transport system, IgG-pretreated jejunal segments were perfused with various other radiolabeled compounds, all at a concentration of 1 mM. The antibody to MFABP induced a 36–53% inhibition of the absorption rate of various long-chain fatty acids, D-monopalmitin, L-lysophosphatidylcholine, and cholesterol. In contrast, uptake of L-alanine remained unaltered. Since radiolabeled short- and medium-chain fatty acids were not available for this study, they could not be included in those antibody inhibition experiments. The above results, together with those of the absorption studies in presence of various unlabeled substrates suggest that this membrane carrier system may have transport competence for long-chain fatty acids, L-lysophosphatidylcholine, monoglycerides, and possibly even for cholesterol. Whether in addition other substrates, e.g., fat soluble vitamins, may also share this transport mechanism remains to be established. The suggestion that the membrane fatty acid carrier system has transport competence for various monomeric lipids needs to be confirmed now by other studies in which the affinity of these lipophilic substrates to the MFABP is determined.

Lack of inhibition of [³H]oleate uptake in presence of equimolar concentration of short- and medium-chain fatty acids is compatible with the hypothesis that not the carboxylic group alone, but the structure of the monomeric hydrocarbon chain may represent the major structural determinant for recognition by the membrane fatty acid transporter (steric or hydrophobic interaction). The indication that also fatty acid methyl ester, D-monopalmitin, and cholesterol may interact with the fatty acid membrane carrier system would support the concept that negative charge is not necessary for uptake.

The suggestion of a common membrane carrier protein, mediating the intestinal absorption of the bulk of lipolytic products generated by pancreatic enzymes represents a new aspect of the overall fatty acid absorption process. Although this is in contrast to the previous assumption of a passive diffusional membrane transport mechanism, it does not exclude that the absorption rate is in addition determined by the diffusion of the fatty acids containing mixed micelles across the unstirred water layer towards the microvillous membrane (30, 31, 35).

Similar to the hepatocellular uptake of fatty acids it was speculated that fatty acid transport across jejunal microvillous membranes might also be driven by an active, Na⁺-dependent, potential sensitive translocation process, which was established in studies with basolateral rat liver plasma membrane vesicles (36). In fact, when mucosal cell uptake of [³H]oleate bound to albumin in a molar ratio of 1:1 was examined in

absence of Na⁺, the initial uptake rates were reduced by 51–61% (Table I). Moreover, pretreatment of mucosal cells with 2 mM ouabain, which is known to inhibit the Na⁺/K⁺-ATPase, reduced the influx rate by 41%, suggesting that uptake might be linked to the activity of this enzyme maintaining the physiologic transmembrane Na⁺/K⁺-gradients. Furthermore, perfusion of the jejunal segment with Na⁺-free medium (isoosmotic replacement of Na⁺ with K⁺) resulted in a 41% inhibition of [³H]oleate absorption. Although those results may be compatible with a Na⁺-dependent transport system, it has to be considered that under those experimental conditions employing isolated mucosal cells and in vivo perfused jejunal segments, it is difficult to differentiate between the requirement of certain ionic gradients and induced electrical potential differences as responsible driving forces. In addition it remains unclear whether those effects are related to the membrane transport machinery or to interactions at the level of other sites of intracellular metabolism. Therefore, it is planned to study the driving forces of fatty acid influx into jejunal mucosal cells in the experimental model of isolated jejunal microvillous membrane vesicles.

Acknowledgments

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