Uptake of Micellar Long-Chain Fatty Acid and sn-2-Monoacylglycerol into Human Intestinal Caco-2 Cells Exhibits Characteristics of Protein-Mediated Transport

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ABSTRACT Long-chain fatty acid and sn-2-monoacylglycerol (2-MG) are the digestive products of dietary triacylglycerol (TG) hydrolysis. Although fatty acid uptake into the enterocyte has been examined widely, less is known about 2-MG uptake, and few studies have mimicked the physiologic conditions present in the postprandial situation. In this study, the cellular uptake of oleic acid and 2-monoolein, presented in taurocholate micellar solution, was examined in human intestinal Caco-2 cells to model the postprandial intestinal milieu. Initial uptake of oleic acid and 2-MG displayed a saturable function of their monomer concentrations, suggesting that fatty acid and 2-MG uptake may be protein-mediated processes at low unbound concentrations of lipid. The initial rate of oleate uptake was faster and the apparent \( K_m \) was lower than values for 2-MG. Unlabeled oleic acid and, to a lesser extent, unlabeled 2-MG, inhibited the uptakes of both \(^{[3]H}\)oleic acid and \(^{[3]H}\)2-monoolein, suggesting competitive uptake. The nonphysiologic isomer sn-1-MG had effects similar to 2-MG, whereas the intermediate digestive product, diacylglycerol (DG), did not inhibit either oleate or 2-monoolein uptake. These results suggest that in the postprandial state, fatty acid and 2-MG derived from dietary TG are transported into the enterocyte, at least in part, via a protein-mediated pathway that is shared by both lipids, but not by the intermediate digestive product, DG.

KEY WORDS: sn-2-MG fatty acid taurocholate postprandial Caco-2 cells

The majority of dietary lipid is triacylglycerol (TG) containing long-chain fatty acids. TG is hydrolyzed primarily by pancreatic lipase in the lumen of the proximal small intestine to produce 2 fatty acids and an sn-2-monoacylglycerol (2-MG). These lipid products are found solubilized in larger aggregates, namely, bile salt mixed micelles and unilamellar vesicles (1). In the normal situation, the micellar phase predominates (2). We showed previously that lipid transport rates from micelles were far more rapid than rates from vesicular phases (3). Thus, the bile salt micelle is the primary lipid milieu from which fatty acid and 2-MG uptake into the absorptive enterocyte occurs. Although the mechanism of fatty acid uptake into cells has been studied extensively (4–7), there are only a few reports that examined cellular 2-MG uptake (8–10).

Studies of lipid uptake in cell culture have typically used lipids solubilized with serum albumin because albumin is the major carrier of lipids in plasma, and binding affinity values are available (11,12). The kinetic analysis of uptake is carried out by monitoring the initial rates of uptake as a function of ligand concentration. Because lipid aggregates such as micelles are not incorporated into cells as intact species (13,14), the monomer concentration, free from the aggregate, is considered the actual ligand. Thus, although bovine serum albumin (BSA) solutions are useful for controlling the lipid monomer concentrations for kinetics studies (12,15,16), such solutions are clearly not an appropriate model of lipid presentation to the enterocyte during intestinal lipid uptake.

In a previous study, the cellular uptake of fatty acid and 2-MG was examined in Caco-2 cells (8). The initial rate of uptake of 2-MG across the apical plasma membrane was found to be a saturable function of the monomer concentration, similar to long-chain fatty acid uptake, in both micellar and BSA solutions. Competitive uptake between fatty acid and 2-MG was observed; however, the experiments were performed using BSA-bound ligand.

The purpose of this study was to examine intestinal diet-derived lipid uptake under conditions that represent the postprandial situation. Thus, lipid digestive products were solubilized in taurocholate micellar solutions, and uptake into well-differentiated Caco-2 monolayers grown on permeable filters was examined.
MATERIALS AND METHODS

Materials. Tritium-labeled oleic acid and triolein were purchased from NEN. Tritiated sn-2-monoolein was obtained from [3H] tritolein by digestion with pancreatic lipase, as previously described (8) and stored at 20°C in hexane. Tritium-labeled sn-1-monoolein was obtained with sn-2-monoolein, as a minor product following pancreatic lipase digestion. Unlabeled oleic acid was purchased from Nu-Chek-Prep. Unlabeled 2-monoolein, 1-monoolein, and diolein were from Serva Research Lab. BSA (fatty acid free), Triton X-100, and pancreatic lipase (type VI) were purchased from Sigma Chemical. Sodium taurocholate (TC) was from Calbiochem. Cell culture media and reagents were obtained from GIBCO.

Cell culture. Caco-2 cells were grown as described previously (4,8). For experiments, cells were plated at a density of 3 x 10⁶ cells/cm² onto polycarbonate Transwell filter inserts with 0.4-μm pores (Costar), and were grown to 14–22 d postconfluence. Cells at passage 40–56 were used.

Preparation of taurocholate-mixed micellar solution. Radiolabeled oleic acid, 2-MG, or 1-MG was dried under N₂. The dried lipids were dissolved in 0.5% (v/v) of ethanol relative to final volume, dispersed in 10 mmol/L TC in PBS, pH 7.4, to obtain the desired concentration, and were further incubated for 1 h at 37°C with shaking at 90 rpm to obtain a homogenous solution. The specific activities of the uptake solutions were 1–20 μCi/mmol for each ligand. For competition studies, unlabeled lipids were mixed with a radiolabeled ligand to obtain the desired ratio. All solutions were used at 37°C for uptake studies.

Determination of unbound lipid concentrations in taurocholate micellar solutions. The monomer concentrations of lipids were determined by the ultrafiltration method described previously (8).

Lipid uptake assay. The initial rates of uptake of the TC-mixed lipids were determined similarly to our previous studies (8). Initial rates of uptake were determined over a range of ligand concentrations to obtain the apparent Michaelis-Menten constant (Kₘ) and maximum velocity (Vₘₐₓ) of uptake using the nonlinear regression analysis by Prism 4 (GraphPad Software) and Woolf plots, as previously described (4). The data were fit to both a saturable (Michaelis-Menten) function and to a combined saturable plus linear function. At low total substrate concentrations, i.e., Kₘ and Vₘₐₓ values obtained from the best fit of the equation to oleate uptake were 4.67 ± 0.57 μmol/L and 2326 ± 193 pmol/mg protein · 10 s, respectively (r² = 0.998, P < 0.01). The Kₘ and Vₘₐₓ values of 2-MG uptake shown in the best fit nonlinear regression were 8.51 ± 2.29 μmol/L and 1456 ± 244 pmol/(mg protein · 10 s), respectively (r² = 0.993, P < 0.01). Woolf plots were also constructed to determine the apparent Kₘ and Vₘₐₓ values for oleic acid and 2-MG uptake when their monomeric concentrations were minimal, as previously reported (8). The monomer concentration of the incubation medium did not change after a 10-s incubation with cells (data not shown). Uptake rates were plotted as a function of unbound monomer concentration of lipid (Fig. 2A). At lower concentrations of unbound ligands, the apical uptake of TC-mixed oleic acid and 2-MG appeared to be a saturable function of the monomer lipid concentration, suggesting that facilitated transport was occurring for both 2-MG and long-chain fatty acid. At higher concentrations, the lipid uptake showed a linear function of the mean concentration of its ligand (Fig. 2A).

FIGURE 1  Monomer concentrations of oleic acid and 2-monoolein in 10 mmol/L TC micellar solution. TC-mixed radiolabeled lipid was filtered, and the radioactivity in the filtrate was estimated as the monomer fraction. Results are means ± SD, n = 3. When no error bars appear, the SD values were smaller than the symbols.

RESULTS

Monomer concentrations of oleic acid and 2-MG in micellar solution. The unbound monomeric concentrations of lipids were estimated by filtration, as described above. Unbound oleic acid in a 10 mmol/L TC micellar solution was consistently 1.6% of the total concentration, up to 250 μmol/L (4.0 μmol/L as the monomer) (Fig. 1). At higher oleic acid concentrations, the proportion of the monomer became lower and unstable. The monomer concentration of 2-MG was 5.0% when the total 2-MG concentration was <125 μmol/L (6.25 μmol/L as the monomer) (Fig. 1). Higher concentration solutions showed lower and unstable unbound levels, similar to oleate. In the case of 1-MG, the proportion of monomer was 3.0% of its total concentration up to 250 μmol/L (data not shown).

The highest monomer concentrations observed within the linear range were similar to the reported CMC values of 6.0 μmol/L for oleic acid (17) and 4.2 μmol/L for 2-MG (8).

Rates of uptake of oleic acid, 2-MG, and 1-MG from micellar solution. The kinetic parameters of uptake were determined using the 10-s point for various concentrations of lipids because the apical uptake of TC-mixed lipids was a linear function of time within 20 s and because metabolism of the lipids was minimal, as previously reported (8). The monomer concentration of the incubation medium did not change after a 10-s incubation with cells (data not shown). Uptake rates were plotted as a function of unbound monomer concentration of lipid (Fig. 2A). At lower concentrations of unbound ligands, the apical uptake of TC-mixed oleic acid and 2-MG appeared to be a saturable function of the monomer lipid concentration, suggesting that facilitated transport was occurring for both 2-MG and long-chain fatty acid. At higher concentrations, the lipid uptake showed a linear function of the mean concentration of its ligand (Fig. 2A). Within the linear range of the lipid monomeric concentrations, the uptake data were analyzed to determine whether they followed the Michaelis-Menten equation or represented a combination of linear and saturable functions. As mentioned earlier, the uptake profiles for 18:1 ≤ 3.2 μmol/L and for 2-MG ≤ 6.5 μmol/L were well fit by the Michaelis-Menten equation (Fig. 2A, dashed lines). The apparent Kₘ and Vₘₐₓ values obtained from the best fit of the equation to oleate uptake were 4.67 ± 0.57 μmol/L and 2326 ± 193 pmol/(mg protein · 10 s), respectively (r² = 0.998, P < 0.01). The Kₘ and Vₘₐₓ values of 2-MG uptake shown in the best fit nonlinear regression were 8.51 ± 2.29 μmol/L and 1456 ± 244 pmol/(mg protein · 10 s), respectively (r² = 0.993, P < 0.01). Woolf plots were also constructed to determine the apparent Kₘ and Vₘₐₓ values for oleic acid and 2-MG uptake when their monomeric concentrations were stable (Fig. 2B). The values obtained for oleate uptake were 3.28 μmol/L and 1851 pmol/(mg protein · 10 s) (r² = 0.905, P < 0.01), and those for 2-MG uptake were 9.77 μmol/L and 1609 pmol/(mg protein · 10 s) (r² = 0.929, P < 0.01) for Kₘ and Vₘₐₓ, respectively. 1-MG uptake appeared similar to 2-MG uptake (Fig. 2A). However, due to the limited amount of radiolabeled lipids available, the 1-MG uptake data were not analyzed.
1-MG available, consistent values for kinetic parameters were not obtained.

**Influence of coexisting lipolytic products on lipid uptake from the micellar solution.** To model intestinal fatty acid and 2-MG uptake under postprandial conditions, uptake was measured in the presence of other lipids that likely coexist in the micelle during dietary lipid digestion. Radiolabeled oleic acid uptake was significantly inhibited by unlabeled oleic acid (Fig. 3A). Inhibition was also observed when 2-MG was added, although the effect was weaker than that of oleic acid. The inhibitory effect of oleic acid addition (52.6 ± 9.4% of control) was significantly stronger than that of 2-MG (78.4 ± 5.4% of control) when their concentrations were 100 times higher than radioactive oleate concentration (P < 0.05). Addition of 1-MG inhibited oleate uptake to a similar extent as 2-MG, whereas DG, an intermediate product of TG digestion, did not have an inhibitory effect.

When radiolabeled 2-MG uptake was examined, unlabeled oleic acid had the strongest inhibitory effect on its uptake, followed by 2-MG and 1-MG (Fig. 3B). The inhibitory effect of oleic acid addition (54.0 ± 4.3% of control) was significantly stronger than that of 2-MG (77.9 ± 15.9% of control) when these unlabeled competitors were added at a concentration 100 times higher than radioactive 2-MG (P < 0.05). Similar to oleic acid, the uptake of 2-MG was not inhibited by DG.

**DISCUSSION**

In this study, uptake rates of the major products of dietary lipid digestion, solubilized in a micellar solution, were investigated using human intestinal Caco-2 cells as a model of the absorptive enterocyte under postprandial conditions. As Hernell et al. (1) demonstrated, the triacylglycerol digestive products, fatty acids and MG, are present not only in a micellar phase but also in other lipid phases, particularly vesicles, in the duodenum. We showed that in vitro, the fatty acid and monocylglycerol transfer rates in micelles are markedly faster than those in unilamellar vesicles; thus, micelles are likely to deliver the majority of the lipid digestive products to the enterocyte (3). Moreover, under normal digestive conditions, the lipids are expected to be present mainly in the micellar phase, due to high luminal bile salt concentrations. Thus, the initial rates of lipid uptake in vivo likely reflect rapid lipid transfer between...
micelles and the enterocyte membrane, the process assessed in the present experiments. To estimate the kinetic parameters of oleic acid and 2-MG uptake, monomer concentrations in 10 mmol/L TC micellar solutions were evaluated by ultrafiltration because it is widely believed that lipid uptake occurs from the soluble monomeric phase (13,14). The unbound concentration of oleic acid in 10 mmol/L TC solution was similar to that reported for other fatty acids (5,6). The concentration of the 2-MG monomer was higher. The constant ratio between the monomer and its total concentration was not observed at higher lipid concentrations, likely due to their exceeding the CMC.

In a previous study, we reported that the $V_{\text{max}}$ values of micellar fatty acid and 2-MG uptake were considerably higher than those of BSA-bound lipid uptake, whereas the $K_{\text{m}}$ values of micellar lipid were lower than those of BSA-bound lipid (8). The $K_{\text{m}}$ values here were higher because the monomeric concentrations of lipid in 10 mmol/L TC micelle had been underestimated previously. Thus, the lipid micelles used in our previous study (8) were of higher total concentration, and the stability of the monomer concentration in the micelle was lower than that in the range of concentrations used in this study. This resulted in a clearly saturated uptake profile because the CMC had likely been exceeded; in the reliable range of concentrations (containing stable monomer concentrations) used herein, the uptake of oleic acid and 2-MG also showed saturable functions of unbound concentrations, although the saturation was not as dramatic. The reestimated micellar $V_{\text{max}}$ values are still considerably higher than the $V_{\text{max}}$ values obtained from BSA-bound lipid uptake, in agreement with others (18,19).

Oleic acid and 2-MG uptake showed a saturable function of their unbound concentrations, supporting the suggestion that dietary lipid uptake into the enterocyte may occur in part by a protein-mediated pathway. The $K_{\text{m}}$ of oleic acid was lower than that of 2-MG, indicating that oleic acid is a more favorable ligand for a putative transmembrane transport protein. In a model system study, it was found that the transfer rate of fatty acid from micellar solutions was faster than that of 2-MG (3). Thus, the delivery of fatty acid for enterocyte, at least in part, via a common facilitated protein-mediated pathway. The $K_{\text{m}}$ values here were higher because the monomeric concentrations of lipid in 10 mmol/L TC micelle had been underestimated previously. Thus, the lipid micelles used in our previous study (8) were of higher total concentration, and the stability of the monomer concentration in the micelle was lower than that in the range of concentrations used in this study. This resulted in a clearly saturated uptake profile because the CMC had likely been exceeded; in the reliable range of concentrations (containing stable monomer concentrations) used herein, the uptake of oleic acid and 2-MG also showed saturable functions of unbound concentrations, although the saturation was not as dramatic. The reestimated micellar $V_{\text{max}}$ values are still considerably higher than the $V_{\text{max}}$ values obtained from BSA-bound lipid uptake, in agreement with others (18,19).

In summary, fatty acid and 2-MG in TC micellar solution showed saturable and competitive uptake into Caco-2 cells. DG, the intermediate digestive product, did not inhibit the uptake of either fatty acid or 2-MG, whereas 1-MG, a structural isomer of 2-MG, had properties similar to 2-MG. These results suggest that under postprandial physiologic conditions, diet-derived fatty acid and 2-MG may be transported into the enterocyte, at least in part, via a common facilitated protein-mediated pathway.
LITERATURE CITED


