Fatty Acid Transfer in Taurodeoxycholate Mixed Micelles†

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ABSTRACT: Dietary triacylglycerol is acted upon by lipolytic enzymes in the stomach and the proximal small intestine, releasing fatty acids and monoacylglycerol as the ultimate products. These digestive products are solubilized by bile released from the gall bladder, resulting in the formation of two product phases—vesicles and micelles—depending upon the concentration of bile in the small intestine. Absorption of lipid is thought to occur from these two phases. We have previously examined the rate and mechanism of long-chain fatty acid transfer between unilamellar vesicles [Kleinfeld, A. M., & Storch, J. (1993) *Biochemistry 32*, 2053−2061]. In order to begin to assess the relative contributions of micellar *vs* vesicular phases in the absorption of dietary lipid, a simple model system was designed to investigate the transfer of fatty acid and monoacylglycerol between micelles. A fluorescence self-quenching assay was used to monitor the transfer of fluorescent anthroyloxy-labeled lipids from donor micelles to acceptor micelles. The mechanism of fatty acid transfer was found to be a combination of diffusional and collisional processes, with the latter dominating at high micelle concentrations. The rate of diffusional transfer of fatty acid and monoacylglycerol analogues was approximately 30-fold greater from micelles than vesicles. Intermicellar and intervesicular rates of transfer were 3-fold greater for fatty acids as compared with monoacylglycerol. The results suggest that uptake of the products of intestinal lipase hydrolysis is more efficient from micellar than vesicular phases. Nevertheless, fatty acid and monoacylglycerol transfer from unilamellar vesicles could account, in part, for the relatively efficient uptake of dietary lipid observed in conditions of intestinal bile salt insufficiency.

The average adult American diet contains approximately 100−150 g of fat/day (Carey et al., 1983). Dietary fat is largely in the form of triacylglycerols (TG),[1] with smaller amounts of other lipids such as phospholipids, cholesterol, and fat-soluble vitamins. TG hydrolysis results in the formation of two major products, monoacylglycerol (MG) and fatty acids (FA). These are thought to be solubilized by bile salts derived from the liver to form a micellar structure in which the polar groups project into the surrounding aqueous phase while the nonpolar hydrocarbon chains form the hydrophobic micellar core. Uptake of FA and MG is then believed to occur from the micelle phase. This micellar hypothesis of lipid absorption, proposed by Hofmann and Borgström (1964), has since been modified to a more complex model of the postprandial intestinal milieu.

Mazer et al. (1980) constructed a phase diagram displaying the coexistence of micelles and vesicles as a function of taurocholate and phosphatidylcholine concentrations which encompass the range of physiologically observed levels. They reported that vesicle formation is favored by a decrease in bile salt concentrations and that progressive dilution of the micellar phase results in the formation of unilamellar vesicles. More recently, it has been reported that the viscous isotropic phase shown previously to arise during *in vitro* lipase digestion (Patton & Carey, 1979) appears to contain liposomal particles as well as micelles (Staggers et al., 1990; Hernell et al., 1990). It was demonstrated that in the postprandial intestine, unilamellar vesicles of variable size and metastability form spontaneously and coexist with micelles. It was further proposed that unilamellar vesicles represent an early product phase of intestinal lipolysis, perhaps originating at the surface of emulsion particles as multilamellar vesicles (Hernell et al., 1990). The bile salt micelles saturated with mixed lipids and cholesterol have an average radius of ≤40 Å and unilamellar vesicles of mixed lipids with bile salt have radii of 200−600 Å (Hernell et al., 1990). It is from these two product phases, namely, micelles and the nonmicelle vesicle phase, that lipid absorption may occur. The physiological importance of the nonmicellar phase is suggested by studies reporting a high index of fat absorption in patients with bile salt deficiency (Carey, 1983) and minimal fat malabsorption in patients with low intraluminal bile salt concentrations (Mansbach et al., 1980).

Mechanisms of fatty acid transfer between unilamellar vesicles have been investigated previously (Storch & Kleinfeld, 1986; Kleinfeld & Storch, 1993). Intervesicular transfer occurs by an aqueous diffusion process, where the fatty acid dissociates from the donor membrane into the aqueous phase prior to association with the acceptor membrane. A similar mechanism appears to describe MG transfer between vesicles.[2] In mixed micelles, only the transfer of fluorescent phospho-

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[1] Abbreviations: TG, triacylglycerol; MG, monoacylglycerol; FA, fatty acid; EPC, egg phosphatidylcholine; AO, anthroyloxy; NBDPE, *N*-(7-nitro-2,1,3-benzoxadiazol-4-yl)phosphatidylethanolamine; PL, phospholipid; TDC, taurodeoxycholate; 12AD, 12-(9-anthroyloxy)dodecanoic acid; 2AP, 2-(9-anthroyloxy)palmitic acid; 16AP, 16-(9-anthroyloxy)palmitic acid; 2AS, 2-(9-anthroyloxy)stearic acid; 12AS, 12-(9-anthroyloxy)stearic acid; 12AO, 12-(9-anthroyloxy)oleic acid; MG12AO, 12-(9-anthroyloxy)-*sn*-1 monooleoylglycerol; C12:0, dodecanoic acid; C16:0, palmitic acid; C18:0, stearic acid; C18:1, oleic acid; IMC, intermicellar concentration.

[2] A. G. Whitney and J. Storch, unpublished observations.

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lipids has been examined (Nichols, 1988), and it was found that phospholipid transferred up to 6000 times faster in micelles than in vesicles. The mechanism of transfer of fatty acids and MG in micelles has not been previously addressed.

In the present study, the rate and mechanism of fatty acid transfer between micelles was examined for the first time. The experiments utilized anthroyloxy analogs of fatty acids in a fluorescence self-quenching assay to measure rates of micellar lipid transfer under various conditions. Micellar and vesicular transfer were compared since these are the two major product phases formed in the intestine. Finally, a comparison between the rates of fatty acid and monoacylglycerol transfer was made, as these are the two primary hydrolytic products of digestive lipase action on dietary lipid. The results demonstrate that transfer from micelles occurs substantially faster than from vesicles and that spontaneous FA transfer rates are somewhat more rapid than MG transfer rates for both micellar and lamellar phases.

MATERIALS AND METHODS

Materials. Egg phosphatidylcholine (EPC) and *N*-(7-nitro-2,1,3-benzoxadiazol-4-yl)phosphatidylethanolamine (NBD-PE) were purchased from Avanti Polar Lipids Inc. (Birmingham, AL). Sodium taurodeoxycholate was purchased from Calbiochem (La Jolla, CA). The following fatty acids were purchased from Nuchek (Elysian, MN): dodecanoic acid (C12:0), palmitic acid (C16:0), stearic acid (C18:0), and oleic acid (C18:1). 1-Monoolein was also from Nuchek. Anthroyloxy-labeled fluorescent analogs were from Molecular Probes (Eugene, OR): 12-(9-anthroyloxy)dodecanoic acid (12AD), 2- and 16-(9-anthroyloxy)palmitic acid (2AP, 16AP), 2- and 12-(9-anthroyloxy)stearic acid (2AS, 12AS), and 12-(9-anthroyloxy)oleic acid (12AO). The *sn*-2 MG is the isomer that is predominantly formed in the intestine, but its instability under experimental conditions causes the formation of the *sn*-1 isomer. Thus the anthroyloxy analogue of the *sn*-1 isomer was used in these experiments. 1-Monoolein labeled with 12AO (MG12AO), prepared by condensation of 12AO with glycerol 1,2-isopropylidene ketal, was prepared by Molecular Probes and the structure was verified by NMR. Less than 10% consisted of the *sn*-2 isomer. Stock solutions of native and fluorescent fatty acids and MG12AO were prepared in ethanol. Stock solutions of 1-monoolein were in hexane. All stock solutions were stored at −20 °C under nitrogen. Buffer solution used for experiments with micelles was 10 mM Tris-HCl/150 mM NaCl at pH 7.4. Vesicles were suspended in 40 mM Tris-HCl/100 mM NaCl at pH 7.4. For experiments with varying temperature, 10 mM Hepes/150 mM NaCl at pH 7.4 was used. All buffer reagents were purchased from Sigma (St. Louis, MO).

Micelle and Vesicle Preparation. Mixed micelles were prepared with lipid and bile salt in the molar ratio of 1.0: 2.0. Eighty percent of the lipid portion was composed of EPC. Twenty percent of the lipid component of acceptor micelles was composed of native fatty acid or monoolein while 20% of the lipid component of the donor micelles was composed of fluorescent fatty acid or monoolein analogs. Sodium taurodeoxycholate was the bile salt used in all studies. Preparation of mixed micelles was by rotary evaporation of solvents followed by overnight lyophilization under vacuum and at low temperature. The dried mixtures were then suspended and if necessary diluted in buffer at the intermicellar concentration (IMC) of bile salt (please see below) (Nichols, 1988). After suspension in buffer, the micellar mixtures were stirred until optically clear. Typically the micellar solutions were found to be almost instantly clear upon stirring. However, micellar solutions were allowed to equilibrate at room temperature for about 45 min before commencement of experiments. All experiments were performed using freshly prepared micellar solutions. As previously described, small unilamellar vesicles (SUV) were prepared using EPC in donors and EPC and NBDPE (10 mol %) in acceptors (Storch & Kleinfeld, 1986).

Measurement of Intermicellar Concentration. The size and number of mixed micelles is dependent on the ratio of the components and the intermicellar bile salt concentration (Mazer et al., 1980). For the determination of the IMC of a given set of micelles, the micelles were mixed with equal volumes of varying dilutions of bile salt in buffer (Nichols, 1988). Light scatter was used to monitor changes in the size of micelles and to determine the IMCs of the various micelles. These experiments were done on the SLM 8000 spectrofluorometer at a constant temperature of 24 °C. The sample was excited at 300 nm and emission of scattered light was detected at 300 nm. Data were collected and plotted over time. The IMC was taken as that concentration of added bile salt that produced no change in light scatter (Nichols, 1988). In the case of micelles containing fluorescent analogs, fluorescence was used as a tool to measure IMC (Nichols, 1988). Samples were excited at 383 nm and emission was detected at 450 nm. Fluorescence changes were monitored over time and the data were collected and plotted to determine the IMC by visual inspection. Similar to the light scatter method, the IMC was determined as the concentration of bile salt that produced no change in fluorescence.

Measurement of Intervesicular Rate of Transfer of Fluorescent Fatty Acids. The transfer of long-chain fatty acids and monoacylglycerol between small unilamellar vesicles was monitored using a fluorescence resonance energy transfer assay, as previously detailed (Storch & Kleinfeld, 1986; Kleinfeld & Storch, 1993) with an Applied Photophysics DX17MV stopped-flow spectrofluorometer. The molar ratio of donors:acceptors was 1:5 in order to ensure unidirectional transfer. The excitation wavelength was 383 nm, and the fluorescence emission was detected using a 408-nm cutoff filter. These experiments were done at a constant temperature of 24 °C. For each separate experimental condition, any decrease in AO fluorescence due to photobleaching was ruled out by mixing donor vesicles with donor vesicles and establishing an unchanging fluorescence signal over time.

The data obtained from the transfer experiment, in the form of individual traces, were best analyzed by a double-exponential fit and an average rate, k_{avg}, was calculated as previously detailed (Storch & Kleinfeld, 1986; Kleinfeld & Storch, 1993). The analysis was performed using a fitting program provided in the Applied Photophysics workstation.

Measurement of Intermicellar Rate of Transfer of Fluorescent Fatty Acids. The self-quenching property of fluorescent fatty acids or monoacylglycerol in the micelles was used to determine the residence time by simply making bulk fluorescence measurements. The residence time of a fatty acid in a micelle is defined as the average time that a fatty acid spends in one micelle. Fresh samples of donors and acceptors were prepared and loaded into syringes on the

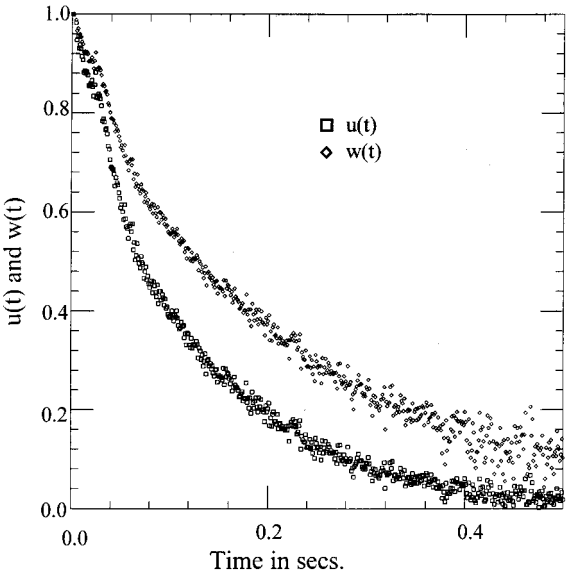

FIGURE 1: Sample plot of intermicellar transfer experimental data. The plot displays the change observed in $u(t)$ and $w(t)$ over the time scale of the mixing experiment. The data are plotted as a scatter plot from one replicate of one experiment using the probe 12AS (18:0) at 24 °C.

Applied Photophysics DX17MV stopped-flow spectrofluorometer. Bleaching tests were done by mixing donor micelles only in order to eliminate any change in fluorescence intensity that was not due to exchange of micellar components. The photobleaching controls were performed prior to each individual experiment. For the transfer assay, equal volumes of donors and acceptors were injected into a mixing chamber and excited at 383 nm. Temperature was maintained using a circulating water bath. Following mixing, the increase in fluorescence, due to the release of self-quenching, was detected using a 408-nm cutoff filter. Final micelle concentrations at $t = 0$ were typically 0.08 mM EPC:0.2 mM NaTDC:0.02 mM AOFA (or MG12AO) for donors and 0.8 mM EPC:2.0 mM NaTDC:0.2 mM native fatty acid (or monoolein) for acceptors. On an average, about 20 traces were obtained for each separate condition.

The fluorescence data, $f(t)$, were recorded over time scales ranging from 0.5 to 30 s depending upon the specific probe. Adequate data were collected both as the change in fluorescence was occurring and after the system was well into equilibrium. The latter was done to obtain an accurate equilibrium fluorescence value for analysis. The relation between the change in fluorescence and the concentration of the fluorescent fatty acid in the micelles was used to analyze the data. Figure 1 depicts the raw fluorescence data, $u(t)$, recorded from the experiment and the transformed data, $w(t)$, obtained from it. The latter was then used in the analysis of the rate constant. A single-exponential fit of the change in concentration of fluorescent fatty acid in the donors over time gave the residence time of the fatty acid in a micelle. The analysis was done using the FORTRAN executable (DECAY.EXE). Details of the analysis are described in the Appendix.

RESULTS

Intermicellar Concentration of Taurodeoxycholate. IMCs of all micelles were determined for each experimental condition. Importantly, the IMC of fluorescent micelles was always found to be the same as their nonfluorescent counterparts. Table 1 is a list of the fatty acids/monoacylglycerol used and the IMCs of the micelles containing them. The IMC for each probe was determined from 2−4 independent trials. In each of the trials, the IMC for a particular probe was found to be exactly the same and therefore no errors are given in the table.

Table 1: Intermicellar Concentration Values of Micellar Solutions Containing EPC, TDC, and FA or MG[a]

probe/lipid	TDC IMC (mM)
lauric acid (12:0)	0.4
12AD	0.4
palmitic acid (16:0)	0.6
2AP	0.6
16AP	0.6
stearic acid (18:0)	0.7
12AS	0.7
oleic acid (18:1)	0.6
12AO	0.6
1-monoolein	0.7
MG12AO	0.7

[a] The IMC of TDC was determined using light scatter and, for the AO probes, light scatter and fluorescence, as described in Materials and Methods.

Mechanism of Fatty Acid Transfer between Micelles. To characterize the mechanism of fatty acid transfer in micelles, the donor to acceptor concentration ratio was varied and the rate constant was determined. A lack of change in transfer rates at these different relative concentrations is taken as an indication of an aqueous diffusional mechanism of transfer. A collisional transfer mechanism is indicated by a first-order increase in transfer rates with increasing acceptor concentration. In these experiments, the acceptor concentration was varied from 5 to 100 times the donor concentration. The rate constants obtained for 12AO transfer did not strictly fit either a diffusional process or a collisional process. Instead, the data were fit to a model previously described by Nichols that splits the transfer process into diffusional and collisional components (Nichols, 1988). Consequently, it was found that with increasing acceptor concentrations there was an increasing predominance of the collisional component over the diffusional component. However, at low acceptor concentrations, the transfer occurred almost entirely via aqueous diffusion. This is depicted in Figure 2 and the corresponding analysis is as follows:

Based on the model of Nichols (1988), the rate constant, k, at a particular acceptor concentration, n_a, can be described by

$$k(n_a)|_{n_d} = k_d \frac{n_a}{n_a + n_d} + k_b \frac{n_a}{n_d} + k_t \frac{n_a^2}{n_d^2} \qquad (1)$$

The first term on the right comes from the diffusion process, and as expected, it approaches a constant as $n_a \gg n_d$. The second term comes from a bimolecular collision process and it is linear in n_a. The third term comes from a termolecular process and it is quadratic in n_a. k_d, k_b, and k_t are the diffusion, bimolecular collision, and termolecular collision rate coefficients, respectively. They all have the same units of reciprocal seconds. These definitions differ slightly from those presented earlier (Nichols, 1988), with the following relationships:

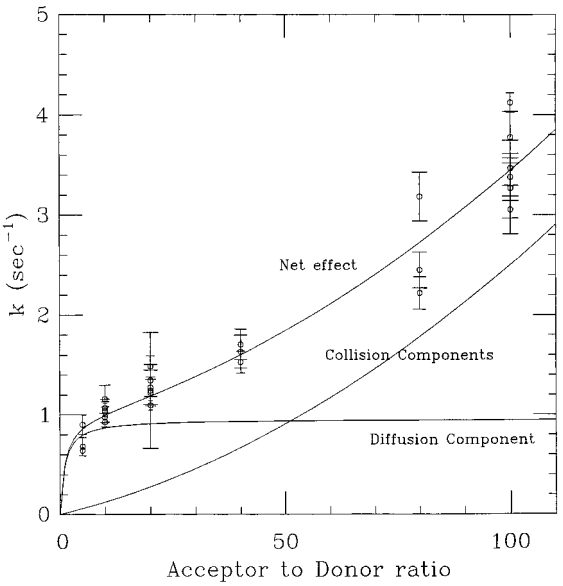

FIGURE 2: Mechanisms of intermicellar transfer of 12AO. The circles with error bars are experimental points at various acceptor to donor molar ratios. A total of 3−6 independent trials were conducted at each acceptor concentration. As per eq 1, the lower two curves represent the contributions of transfer via aqueous diffusion and via collision to the net transfer rate k at a given acceptor micelle concentration. The uppermost curve represents the sum of these two components.

$$k_{dis} = k_d \qquad k_{bi} = \frac{k_b}{n_d} \qquad k_{ter} = \frac{k_t}{n_d^2} \tag{2}$$

where k_{dis}, k_{bi}, and k_{ter} are the definitions in Nichols (1988).

A typical trial had more than three acceptor concentrations for which $k(n_a)$ was determined. The method of least squares was used to calculate the three coefficients in eq 1. From the trials, this analysis yielded the following best fit for the three rate coefficients: $k_d = 0.9542\ s^{-1}$, $k_b = 0.01124\ s^{-1}$, and $k_t = 0.000138\ s^{-1}$. At a bulk solution donor micelle lipid concentration of 0.1 mM, the constants in eq 2 are $k_{dis} = 0.9542\ s^{-1}$, $k_{bi} = 1.124 \times 10^2\ s^{-1}\ M^{-1}$, and $k_{ter} = 1.38 \times 10^4\ s^{-1}\ M^{-2}$, where the molar units refer to the bulk solution concentration of lipid in the acceptor micelles.

Effect of Fatty Acid Structure on Intermicellar Transfer. In order to study the effet of the unsaturation of fatty acids upon their rate of transfer between micelles, a comparison of fully saturated stearic acid (18:0) and monounsaturated oleic acid (18:1) probes was performed. In these experiments, donor micelles containing 20% 12AO or 12AS and acceptor micelles containing 20% oleate or stearate were prepared. At donor to acceptor ratios of 1:10, it was found that 12AS had a transfer rate that was about 3-fold slower than 12AO. However, at donor to acceptor ratios of 1:100, these differences between 12AO and 12AS were absent (Figure 3).

The effect of fatty acid chain length on intermicellar transfer rates was investigated by comparing 2AP (16:0) with 2AS (18:0), and 12AD (12:0) with 16AP (16:0). To control for position of probe attachment (Storch & Kleinfeld, 1986), the anthroyloxy group was attached at the second position from the fatty acid carboxyl group in 2AP and 2AS and at the position farthest from the carboxyl group in 12AD and 16AP. 2AP *vs* 2AS showed the effect of an increase in chain length of two carbon atoms, while 12AD *vs* 16AP showed

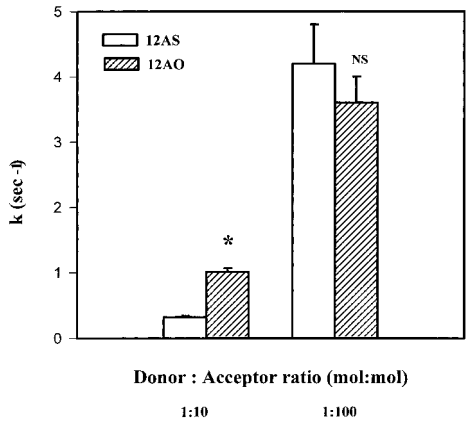

FIGURE 3: Effect of fatty acid monounsaturation on intermicellar transfer rate. Comparison of 12AS (18:0) and 12AO (18:1) transfer rates, k, at two different donor to acceptor ratios. Donor micelle lipid concentration was 0.1 mM. Results are an average of three independent trials. *, significant difference with $p = 0.003$. NS, not significantly different.

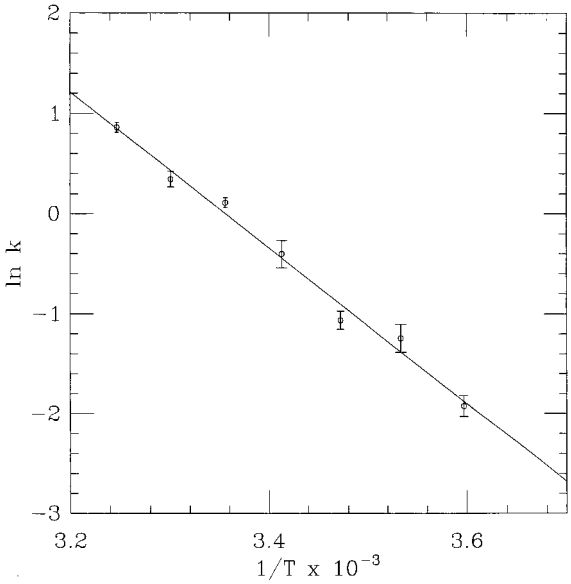

FIGURE 4: Effect of temperature on AOFA intermicellar transfer. Arrhenius plot of the micellar 12AO transfer data obtained at temperatures ranging from 5 to 35 °C. The fit is a linear regression. Each point is obtained from three independent trials.

the effect of an increase of four carbon atoms in chain length. The addition of two carbon atoms from 16:0 to 18:0 resulted in a small but reproducible decrease in transfer rate from $0.12 \pm 0.01\ s^{-1}$ for 2AP to $0.10 \pm 0.04\ s^{-1}$ for 2AS. This difference was not found to be significant. The addition of four carbon atoms from 12:0 to 16:0 resulted in a dramatic 45-fold decrease in transfer rate from $37.0 \pm 4.2\ s^{-1}$ for 12AD to $0.82 \pm 0.07\ s^{-1}$ for 16AP ($p = 0.006$).

Effect of Temperature. To determine the thermodynamic parameters of the intermicellar transfer process, the rate of transfer of 12AO was measured at temperatures from 5 to 35 °C in steps of 5 °C. The donor to acceptor ratio was maintained at 1:10. The rate constants were determined at each temperature, and the results are shown as an Arrhenius plot in Figure 4. The activation energy, E_a, was obtained from the slope of the regression (Eisenberg & Crothers, 1979). The Eyring rate theory was utilized to obtain the thermodynamic potentials from the data at 25 °C (Kleinfeld & Storch, 1993), and these are listed in Table 2. The $\Delta G^{\ddagger}$

Table 2: Thermodynamic Potentials of Intermicellar Transfer[a]

E_a	15.4 ± 0.5 kcal/mol
$\Delta H^{\ddagger}$	14.8 ± 0.5 kcal/mol
$T\Delta S^{\ddagger}$	−2.5 ± 0.5 kcal/mol
$\Delta G^{\ddagger}$	17.3 ± 0.1 kcal/mol

[a] Thermodynamic potentials for the transfer of 12AO were calculated using the Eyring rate theory. The values are the average of three independent trials estimated at a temperature of 25 °C.

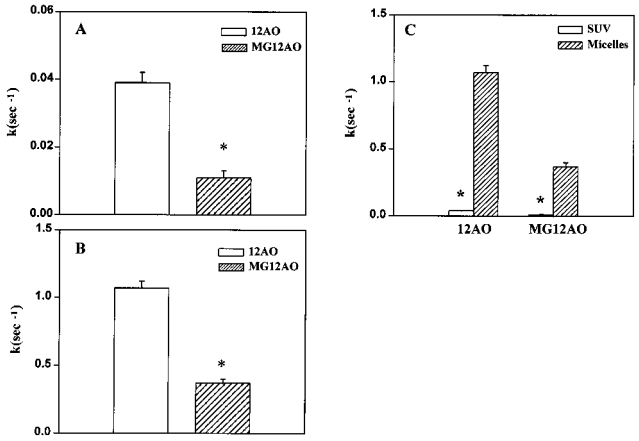

FIGURE 5: FA and MG transfer between vesicles and between micelles. Comparison of 12AO and MG12AO transfer rates, *k*, in (A) vesicles and (B) micelles at a donor:acceptor ratio of 1:10. Donor micelle lipid concentrations were 0.1 mM. Results are from three independent trials. *, significant difference: (A) $p = 0.04$, (B) $p = 0.003$. (C) Comparison of 12AO and MG12AO transfer rates, *k*, in micelles *vs* vesicles. Data from panels A and B are replotted. *, significant difference, $p = 0.001$ for FA and $p = 0.004$ for MG.

of the transfer process was 17.3 kcal/mol, which comprised a large enthalpic component and a smaller $T\Delta S^{\ddagger}$ component.

Micellar vs Vesicular Transfer of Fatty Acid and Monoacylglycerol Analogues. The probes 12AO and MG12AO were used to compare the rates of intermicellar transfer of fatty acid *vs* monoacylglycerol at 24 °C. Acceptor micelles contained 20% oleate or 1-monoolein, respectively. It was found that the monoacylglycerol analogue, MG12AO, transferred about 3-fold more slowly than the corresponding fatty acid analogue, 12AO. This magnitude of difference was seen for both intermicellar and intervesicular transfer (Figure 5A,B). Direct comparison of lipid transfer in micelles *vs* vesicles (data replotted in Figure 5C) revealed that intermicellar rates of transfer of both FA and MG species were 30-fold faster than the corresponding intervesicular rates, under the same conditions.

DISCUSSION

The exchange of molecules between membranes serves to transfer materials, energy, or information between them (Jähnig, 1984). Intermembrane transfer of lipids has been extensively investigated (Dawidowicz, 1987; Brown, 1992). Mixed micelles form the major solubilized lipid phase present in the small intestine during lipid digestion. Fatty acids and monoacylglycerol, the two major products of dietary lipid digestion, aggregate along with bile salts such that absorption can occur efficiently. Although the transfer of phospholipids in micelles has been studied (Nichols, 1988; Fullington et al., 1990), the kinetics of intermicellar fatty acid and monoacylglycerol transfer have not as yet been examined. The studies presented here begin to characterize the process of fatty acid transfer in mixed micelles and, to a lesser extent, the transfer of monoacylglycerol.

Fluorescent anthroyloxy fatty acids were used to label the micelles and to monitor transfer. These fluorescent probes have been used previously to investigate the structural properties of micelles, vesicles, and biological membranes (Thulborn et al., 1979; Storch & Schachter, 1985; Storch & Kleinfeld, 1986). The presence of the large hydrophobic anthroyloxy group will undoubtedly affect the rates of transfer and these will not reflect the actual rates of transfer of native fatty acids. Nevertheless, the relative relationships observed will likely remain the same as those for native fatty acids.

Intermembrane transfer of many types of lipids has been found to occur through aqueous diffusion, where the hydrophobic ligand dissociates from the donor membrane, enters into the bulk aqueous phase, and associates with the acceptor membrane (Brown, 1992). An alternative intermembrane transfer mechanism is a collisional process, in which the rate of transfer is directly proportional to (though not necessarily equal to) the number of collisions and therefore to the number of acceptors present (Brown, 1992). A standard procedure used to distinguish these mechanisms is to determine the transfer rate constant as a function of increasing acceptor concentrations. If the process were wholly diffusional, no change in rate constants would be expected. If the process was collisional, a first-order increase in rates with increasing acceptor concentration would be found. We observed that in a manner very similar to that proposed for phospholipid transfer in micelles (Nichols, 1988), AOFA transfer in micelles fit a model that was comprised of both diffusional and collisional processes. At low acceptor levels, till about 10-fold greater acceptor relative to donor concentration, the transfer was dominated by the aqueous diffusion process. As the acceptor concentration increased, the collisional component began to dominate over the diffusional component, which became a constant. At acceptor micelle concentrations of 100-fold greater relative to donor concentration, AOFA transfer was mostly collisional. The differences in the effect of fatty acid unsaturation on transfer rates at low *vs* high acceptor:donor ratios (Figure 3) provides strong support for the hypothesis of collisional transfer at high micelle concentrations and diffusional transfer at low micelle concentrations. At an acceptor:donor micellar ratio of 10:1, monounsaturated fatty acids transferred 3-fold more rapidly than saturated fatty acids under the same experimental conditions. This can be attributed to differences in aqueous solubilities, with unsaturation increasing the solubility of the fatty acid and therefore increasing its rate of transfer (Storch & Kleinfeld, 1986; Massey et al., 1982). It was also found that the differences in transfer rates between 18:0 and 18:1 analogues disappeared at high acceptor concentrations (i.e., donor:acceptor ratio of 1:100), suggesting that fatty acid aqueous solubility is an important factor determining transfer rates at low lipid concentrations but that it is not a major regulatory factor at high lipid concentrations, where collisional transfer may predominate. In the normal postprandial intestine, then, it is predicted that fatty acids and perhaps monoacylglycerol transfer would occur via collisional transfer. Whether direct collisional interactions with the enterocyte plasma membranes occur remains to be determined.

Fatty acid chain length has also been found to modulate transfer in unilamellar vesicles. Storch and Kleinfeld (1986) reported small differences (2-fold) when comparing palmitate (16:0) with stearate (18:0) analogues and very large differences (1−2 orders of magnitude) between short-chain fatty acids with chain lengths of 11 and 12 carbons and long-chain fatty acids having 16 and 18 carbon atoms. Fullington and Nichols (1993) reported that decreasing the acyl chain length of phospholipids dramatically increased the rate of NBD-labeled phospholipid transfer in taurocholate/phosphatidylcholine mixed micelles. The differences seen here for micellar rates of fatty acid transfer are qualitatively very similar, in that shorter chain length fatty acids tended to transfer more rapidly than longer chain length fatty acids. Here too, this change was not found to have a linear relationship with the absolute chain length. Transfer of the C12 analog was more than 40-fold faster than that of a corresponding C16 fatty acid, while transfer of a 16-carbon anthroyloxy fatty acid was only about 25% faster than the corresponding 18-carbon fatty acid. These differences may be attributed to differences in fatty acid aqueous solubilities, as these are not linearly related to chain length (Storch & Kleinfeld, 1986; Shinoda & Freiberg, 1986). It is also possible that the short-chain fatty acids, perhaps analogous to their interactions with bilayers, are more loosely associated with the micelle (Chalpin & Kleinfeld, 1983). For example, they may be associated with the micelle surface, whereas the longer fatty acid chains might be intercalated between the phospholipid acyl chains. This could cause easier and more rapid dissociation of short chain length fatty acids from the micelle.

A comparison of lipid transfer rates from micelles *vs* vesicles revealed that both fatty acid and monoacylglycerol transfer at least 30-fold faster from micelles than from vesicles. As mentioned above, low bile salt concentrations favor vesicle formation (Hernell et al., 1990; Staggers et al., 1990). It is thus interesting to note that studies in intact animals have shown 60% absorption of fat in the absence of bile (Borgström, 1953; Gallagher et al., 1965). Furthermore, when bile fistula rats were used to simulate a reduced intestinal lipid solubilization milieu (i.e., reduced bile salt milieu), it was found that relatively good absorption of FA and MG occurred, with greater absorption of the former. The presence of an alternative nonmicellar mechanism for lipid dispersion was suggested (Morgan & Borgström, 1969). The present results show that transfer is slower from vesicles, and thus provides a mechanism which could, in part, explain the slower lipid absorption during relative bile salt insufficiency. Nichols (1988) has shown that phospholipid transfer is 200−6000 times faster from PC-TC micelles than from PC vesicles. Differences of this order for FA and MG transfer might have been expected to dramatically inhibit lipid absorption. Our results, however, show that micellar transfer of FA or MG is as little as 30-fold slower in vesicles than in micelles, thereby supporting the physiological observation that in conditions of low bile salt, where the vesicle phase dominates the postprandial luminal milieu, FA and MG assimilation occur to an adequate extent. Interestingly, Hoffman and Hofmann (1973) had shown that oleic acid was absorbed to a greater extent from a micellar solution solubilized by taurocholate than from an emulsion phase stabilized with gum acacia and that a larger portion of the intestine was involved in lipid absorption in the absence of bile and micelles. Although a vesicular phase was not examined, such an increase in anatomical absorptive area may take place in the presence of vesicles as well.

Fatty acid transfer was 3-fold faster than its corresponding monoacylglycerol from both micelles and vesicles, implying that the monoacylglycerol may not be absorbed at the same rate as the fatty acid. Indeed, Morgan and Borgström (1969) showed that fatty acids were absorbed more efficiently than monoacylglycerol. In this context, the importance of bile salt-activated lipase in the infant becomes clear as this enzyme may completely hydrolyze triacylglycerol to yield 3 fatty acids/mol, thereby ensuring the infant of efficient lipid absorption (Bernback et al., 1990).

Estimation of the thermodynamic potentials which describe the intermicellar transfer process for AOFA showed a relatively low $\Delta G^{\ddagger}$ value, which could in part explain the relatively high rate constants for fatty acid transfer in micelles relative to vesicles. The $\Delta G^{\ddagger}$ for intermicellar fatty acid transfer was 5−7 kcal/mol lower than those obtained for FA transfer from small and large unilamellar vesicles (Kleinfeld & Storch, 1993) and is within the range of free energies calculated for spontaneous intermembrane transfer of a variety of amphipathic lipids (Zucker et al., 1992). A decrease in $T\Delta S^{\ddagger}$ was found to characterize the intermicellar transfer process, qualitatively similar to that observed for intervesicular AOFA transfer (Kleinfeld & Storch, 1993). The movement of a hydrophobic ligand through the aqueous phase is thought to order the water molecules during the transfer process, hence resulting in a decrease in entropy. The present results were obtained at a donor:acceptor ratio of 1:10, where, as previously discussed, an aqueous diffusion transfer mechanism is proposed to predominate.

These experiments were performed to further our understanding of the mechanisms of intestinal lipid digestion and absorption, with particular reference to micellar lipid transfer. Model systems that examined simple variables were constructed. In the intact organism, the scenario is of course far more complex. A variety of lipids, including cholesterol and the fat-soluble vitamins, certainly influence the physicochemical properties of the micelles and the vesicles. In addition, the digestive content in the intestine would simultaneously contain both micelle and vesicle phases (Hernell et al., 1990; Staggers et al., 1990), which would also affect net transfer rates. Yet the comparative rates and mechanisms of these transfer processes observed *in vitro* are likely to be valid. In addition, the present results provide a mechanistic framework which can help explain a number of observations from previous physiological studies of lipid absorption. These include the relative rates of fatty acid and monoacylglycerol uptake (Morgan & Borgström, 1969), the relative rates of lipid absorption from micellar and nonmicellar solutions, and the well-known adequacy of lipid uptake under conditions of bile salt insufficiency (Carey, 1983; Mansbach et al., 1980).

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APPENDIX

The fluorescence emitted by a micelle depends upon the fraction of fluorescent fatty acid present in the micelle. For concentrations in the range of 0−20 mol %, the relationship is well described by the Stern−Volmer equation (Lakowicz, 1983):

$$f = \frac{f_0 r Q}{1 + \mu r} \tag{A1}$$

where f is the total fluorescence emitted, r is the fraction of fluorescent fatty acid in one micelle, Q is the total number of lipid molecules in a micelle, f_0 is a proportionality constant, and μ is the quenching constant.

To determine the rate of fatty acid transfer from one micelle to another, two types of micelles are used—donors and acceptors. Donors contain fluorescent fatty acid while acceptors contain the same fraction of native fatty acid. Lipid exchange occurs when these micellar solutions are mixed. Fluorescent and native fatty acids distribute themselves equally among donors and acceptors. With time, the proportion of fluorescent fatty acid decreases in the donors and increases in the acceptors. When the reaction mixture has reached equilibrium, all micelles contain the same fraction of fluorescent fatty acid.

Let n_d be the number of donor micelles, n_a be the number of acceptor micelles, and the total number of micelles $N = n_a + n_d$. Let $r_d(t)$ be the fraction of fluorescent fatty acid in one donor micelle and $r_a(t)$ be the fraction of fluorescent fatty acid in one acceptor micelle at any time t. The initial conditions of the experiments are

$$r_d(0) = r_0 \qquad r_a(0) = 0 \tag{A2}$$

Since there are Q lipid molecules in a micelle and n_d donor micelles, the total amount of fluorescent fatty acid is equal to

$$(r_0 Q) n_d \tag{A3}$$

and this remains unchanged throughout the experiment. At equilibrium, this amount of fluorescent fatty acid is equally distributed among N micelles. Therefore, there are $r_0 Q n_d / N$ fatty acids/micelle and at equilibrium

$$r_d(\infty) = r_a(\infty) = \frac{r_0 n_d}{N} \tag{A4}$$

From the Stern−Volmer equation (A1), initial fluorescence, i.e., at $t = 0$, arising from n_d donor micelles having r_0 fraction of fluorescent fatty acid is

$$f(0) = \frac{f_0 Q r_0}{1 + \mu r_0} n_d \tag{A5}$$

Fluorescence at equilibrium, i.e., at $t = \infty$, arising from N having $r_0 n_d / N$ fraction of fluorescent fatty acid is

$$f(\infty) = \frac{f_0 Q r_0 M}{1 + \mu r_0 M} N \tag{A6}$$

where

$$M = \frac{n_d}{N} \tag{A7}$$

and μ, as mentioned earlier, is the quenching constant. F_r, the ratio of $f(\infty)$ and $f(0)$, is

$$F_r = \frac{f(\infty)}{f(0)} = \frac{1 + \mu r_0}{1 + \mu r_0 (n_d / N)} \tag{A8}$$

Since the value of μ is not equal to 0, due to the presence of self-quenching, $F_r \neq 1$ and there is a change in fluorescence observed in the mixing reaction. This change, as a function of time, can be used to measure the residence time of a fluorescent fatty acid in a micelle.

As can be seen from eq A8, F_r depends experimentally on the ratio of n_d:N, i.e., the ratio of the donors to total micelles. The smaller this ratio, the larger the difference between the final and initial fluorescence, or in other words, the greater the fluorescence change. An appreciable difference between f_∞ and f_0 is also essential for a good signal which would then enable more accurate calculation of the rate constant from the experimental data. Thus, as a standard procedure in all experiments, this ratio was maintained at 1:11 (i.e., the number of acceptors was 10 times greater than the number of donors). This was sufficient to see a good difference in initial and final fluorescence.

F_r also depends on the fraction r_0. As r_0 increases, F_r also increases. Therefore, as large a value for r_0 as possible is picked, while still making sure that it obeys the Stern−Volmer equation. To experimentally determine this fraction, micelles containing varying proportions (0−60%) of fluorescent fatty acid were prepared. The sample containing no fatty acid was considered to be the blank. The fluorescence intensities were plotted against the proportion of fluorescent fatty acids present in the micelle samples and the value of r_0 was determined by visual inspection. This experiment was performed for each of the probes used. With all the probes, 20% was found to be the proportion of fluorescent fatty acid in the micelles that exhibited the maximum fluorescence while still obeying the Stern−Volmer relation.

Data Analysis. The total fluorescence at any time t, $f(t)$, is the sum of the fluorescence from the donors and the fluorescence from the acceptors, each given by the Stern−Volmer equation (A1):

$$f(t) = \frac{f_0 r_d}{1 + \mu r_d} n_d + \frac{f_0 r_a}{1 + \mu r_a} n_a \tag{A9}$$

The total number of fluorescent fatty acids in the mixture is the same at all times and this yields the following relation between $r_a(t)$ and $r_d(t)$:

$$r_a(t) Q n_a + r_d(t) Q n_d = r_0 Q n_d \tag{A10}$$

The left-hand side is the expression for the total number of fluorescent fatty acids at any time t, the first term in the sum being the one coming from the acceptor and the other coming from the donor. The right-hand side is from eq A3. These two equations, A9 and A10, can be solved for $r_d(t)$. The result is the following quadratic equation:

$$p_1 w^2 + p_2 w + p_3 = 0 \tag{A11}$$

where

$$p_1 = F_r(1 - F_rM) + (F_r - 1)^2Mu,$$
$$p_2 = (F_r - 1)(2M - 1)u, \quad \text{(A12)}$$
$$p_3 = (M - 1)u$$

and

$$u = \frac{f(t) - f(\infty)}{f(0) - f(\infty)}$$
$$w = \frac{r_d(t) - r_d(\infty)}{r_d(0) - r_d(\infty)} \quad \text{(A13)}$$

F_r is given by eq A8 and can be determined from the fluorescence observed at the beginning and at the end of the reaction. M is a ratio given by eq A7 and is also known in each experiment. $u(t)$ is a quantity that can be calculated for every time point, t, during the reaction. It directly uses the fluorescence data obtained at the beginning, the end, and during the mixing reaction. $w(t)$ can be obtained from eq A11 by using experimental values for $u(t)$, F_r, and M. Solving eq A11 yields two possible roots for w. The relevant solution is

$$w = \frac{-p_2 + (p_2^2 - 4p_1p_3)^{1/2}}{2p_1} \quad \text{(A14)}$$

Figure 1 is a sample plot showing the change in $u(t)$ and $w(t)$ over time. The rate of decay of $w(t)$ is equal to the rate of decay of $r_d(t)$ (refer to eq A13). From eq A13 we see that $w(0) = 1$ and $w(\infty) = 0$. Therefore

$$w(t) = e^{-kt} \quad \text{(A15)}$$

where $t_{1/2} = 1/k$ is the residence time of the fatty acid in a micelle, the desired quantity.

The executable (DECAY.EXE) implements the above equations (A11−A13) to obtain $w(t)$, which is then fitted to give k. This analysis was initially tested by its utilization to measure the rates of transfer of NBD-labeled phosphatidylethanolamine of varying acyl chain lengths. The results obtained were found to be consistent with the differences found in similar experiments of Nichols (1988). In a typical experiment, approximately 20 replicates were analyzed for each individual sample. k, the rate constant, is obtained by the method of least squares.

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