JPP Journal of Pharmacy and Pharmacology

JPP 2002, 54: 809–819

Received October 2, 2001
Accepted February 5, 2002
ISSN 0022-3573

Effect of dietary fatty acids on the intestinal permeability of marker drug compounds in excised rat jejunum

D. F. Vine, S. A. Charman, P. R. Gibson, A. J. Sinclair and C. J. H. Porter

Abstract

The aim of this study was to explore the effects of diets containing saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and ω-3 and ω-6 polyunsaturated fatty acids (ω-3 and ω-6 PUFA, respectively) on the passive and active transport properties of rat jejunum using marker compounds. Rats were fed diets supplemented with 18.4% (w/w) lipid (4 groups) or standard rat chow (1 group) for a period of 30 days. At the end of the dietary period, mucosal scrapings were taken for the determination of membrane phospholipids, and the apparent jejunal permeability of radiolabelled marker compounds was determined using modified Ussing chambers. Changes in the phospholipid content of the brush border membrane reflected the different lipid content of the diets. The passive paracellular permeability of mannitol was not significantly affected by the fatty acid composition of the diet, although there was a trend toward decreased mannitol permeability in the rats fed both the ω-3 and ω-6 PUFA diets. In comparison, the transcellular diffusion of diazepam was reduced by 20% ($P < 0.05$) in rats fed diets supplemented with ω-3 and ω-6 PUFA. In the lipid-fed rats, the serosal to mucosal flux of digoxin, an intestinal P-glycoprotein substrate, was reduced by 20% ($P < 0.05$) relative to the chow-fed group, however there were no significant differences between the different lipid groups. The active absorption of D-glucose via the Na^+-dependent transport pathway was highest in the SFA, MUFA and PUFA ω-3 dietary groups, intermediate in the low-fat chow group and lowest in the PUFA ω-6 group, and was positively correlated with short-circuit current. These studies indicate that dietary fatty acid changes can result in moderate changes to the active and passive transport properties of excised rat jejunum.

Department of Pharmaceutics, Victorian College of Pharmacy, Monash University, Parkville, Victoria, Australia

D. F. Vine, S. A. Charman, C. J. H. Porter

University of Melbourne Department of Medicine, The Royal Melbourne Hospital, Melbourne, Victoria, Australia

P. R. Gibson

Department of Food Science, Royal Melbourne Institute of Technology University, Melbourne, Victoria, Australia

A. J. Sinclair

Correspondence: C. J. H. Porter, Department of Pharmaceutics, Victorian College of Pharmacy, Monash University, Parkville, Victoria, Australia. E-mail: chris.porter@vcp.monash.edu.au

Acknowledgement and funding: We would like to thank Dr Duo Li (Department of Food Science, RMIT, Melbourne, Australia) for the fatty acid analysis. This study was funded by the Special Monash University Research Fund. The support of an American Association of Pharmaceutical Scientists (AAPS) New Investigator Grant to C.J.H.P. is also acknowledged.

Introduction

The role of dietary lipids in health and disease is well established and has led to a recommendation by world health organizations that the amount of fat in the diet be reduced to 15–30% of the total caloric content of the diet. Based on epidemiological evidence, it has been further suggested that the amount of saturated fat in the diet be reduced to less than 10% of the daily caloric content, with a recommended increase in the intake of polyunsaturated fatty acids (PUFA) and monounsaturated fatty acids (MUFA) (NHMRC 1992).

Dietary lipid changes have been shown to influence the composition and function of cell membranes, including the activity of membrane-bound enzymes and transporters (Thomson et al 1986; Wild et al 1997). In the brush border (BBM) and basolateral (BLM) membranes of the small intestine, dietary fatty acids are

incorporated into the phospholipid acyl chains and may promote differential effects on electrogenic response (Cartwright-Shamoon et al 1995; Lindley et al 1995), membrane fluidity (Brasitus et al 1985, 1989), enzyme activity and nutrient uptake (Brasitus et al 1985, 1989; Thomson et al 1986, 1988). Dietary fatty acids have also been shown to be important in modulating nutrient absorption in intestinal disorders such as Crohn's disease, ulcerative colitis and diabetes (Thomson et al 1996; Burke et al 1997; Miura et al 1998; Endres et al 1999).

The fatty acid composition of the BBM is highly regulated in order to maintain intestinal membrane function, however previous data suggest that feeding diets rich in certain fatty acids leads to specific changes in the fatty acid composition of the membrane phospholipids. The effects of these changes on intestinal transport properties are potentially twofold. First, the overall fluidity of the BBM, or the fluidity of microdomains surrounding membrane-bound proteins, may be altered, which might be expected to affect the permeability of substances across the membrane or alter the affinity of membrane-residing transporters for their substrates. For example, previous studies have noted a correlation between changes in BBM phospholipid fatty acids and altered nutrient uptake (e.g. amino acids, fatty acids, glucose) into BBM vesicles and intestinal segments (Thomson et al 1986; Brasitus et al 1989). Further reports have also described a decrease in the non-receptor-mediated transcellular flux of horseradish peroxidase across Caco-2 cells after cells were pre-incubated with linoleic acid (18:2 ω-6) and eicosapentaenoic acid (20:5 ω-3) (Rosella et al 2000). Second, polyunsaturated fatty acids are known to act as intracellular secondary messengers and may modify the post-transcriptional expression and activity of intestinal membrane-bound enzymes and transporters (Proulx 1991; Thomson et al 1996). These previous studies, however, have generally examined tissue accumulation or uptake into BBM vesicles, methods that in both cases preclude an assessment of the dynamic absorptive and secretory transport properties of the intestine.

The known impact of dietary lipid changes on certain aspects of membrane structure, fluidity and transport (Brasitus et al 1985, 1989; Thomson et al 1987b, 1988), and the potential therapeutic benefit of diets rich in specific fatty acids (Thomson et al 1996; Endres et al 1999), stimulated the current study into the impact of alterations to dietary lipid (dietary fatty acid composition) on intestinal drug permeability. The aim of the study was to compare the effects of pre-feeding different dietary lipids on the intestinal permeability of selected marker compounds. Markers were chosen to reflect the active and passive processes that facilitate drug transport across the small intestine. Rats were fed either a standard chow diet or diets enriched with saturated fatty acid (SFA), MUFA, ω-3 PUFA and ω-6 PUFA, and the apparent intestinal permeability (P_{app}) of marker compounds across rat jejunal tissue was assessed in-vitro using modified Ussing chambers. The passive transport markers, mannitol and diazepam, were used to probe paracellular and transcellular pathways, while active transport and efflux processes were assessed using D-glucose and digoxin, respectively.

Materials and Methods

Materials

The radiolabelled markers [^{14}C]mannitol (specific activity 51.5 mCi mmol^{-1}), [^{3}H]diazepam (sp. act. 83 Ci mmol^{-1}), [^{3}H]digoxin (sp. act. 19 Ci mmol^{-1}) and [^{14}C]D-glucose (sp. act. 54 mCi mmol^{-1}) were purchased from NEN Life Science Products (Boston, MA). Lipids used for dietary supplementation included butter (Meadow Lea, Melbourne, Australia), olive oil (Vetta, Sydney, Australia), safflower oil (Puroil, Dubbo, Australia) and Maxepa fish oil (RP Scherer Melbourne, Australia). Lanoxin injection (250 μg digoxin, 80 mg ethanol, 415 mg propylene glycol, 0.69 mg citric acid (anhydrous), 1.79 mg sodium phosphate and water for injection to 1 mL) was obtained from Sigma Pharmaceuticals (Melbourne, Australia). All organic solvents and chemicals used were of the highest analytical grade. Water was obtained using a Milli-Q water purification system (Millipore, Bedford, MA).

Animals and diets

All animal studies were performed in accordance with the Australian and New Zealand Council for the Care of Animals in Research and Teaching guidelines and the study protocol was approved by Monash University institutional animal experimentation ethics committee. Male Sprague Dawley rats (100–150 g) were fed isocaloric, semi-synthetic diets formulated in accordance with National Research Council (1995) criteria. Control animals were fed standard rat chow containing 5.2% (w/w) lipid, 18.9% protein, 5% fibre and 70.9% carbohydrate (Glen Forrest Stockfeeders, Glen Forrest, Australia). Experimental animals were fed diets containing 18.4% (w/w) lipid, 18% (w/w) protein (casein), 44% (w/w) cornstarch, 11% (w/w) sucrose, 5% (w/w)

fibre, 1% (w/w) vitamin mix (supplied by Propharma, Dandenong, Australia), 3.5% (w/w) mineral mix (AIN mineral mix 93-G was prepared as recommended by NHMRC 1997 guidelines), 0.1% (w/w) choline chloride and 0.3% (w/w) methionine. The diets were supplemented with different lipid sources including SFA (butter), MUFA (olive oil), ω-3 PUFA (fish oil) and ω-6 PUFA (safflower oil). Diets were freshly prepared every two weeks, with the exception of the fish oil diet, which was prepared weekly to avoid oxidation. All diets were stored at -20°C before use and all animals were fed for a period of 30 ± 1 days. Animals were given free access to food and water, and were housed two rats to a cage with a 12-h light–dark cycle according to NHMRC animal housing recommendations (NHMRC 1997). Food was changed daily and animal weights, food intake and stool output were recorded for a 24-h period every 3 days. At the end of the 30-day feeding period and on the day before killing, animals were fasted for a period of 18 h before tissue excision for permeability studies.

Permeability studies

The permeability of radiolabelled marker compounds was assessed using a modified Ussing chamber procedure as previously described (Ungell et al 1992). Briefly, animals were anaesthetized with isofluorane and subsequently killed using a lethal dose of sodium pentobarbitone (750 mg/rat) administered to the heart. A midline abdominal incision was made and the jejunum was removed distal to the ligament of Treitz, immediately placed in ice-cold Krebs buffer supplemented with sodium L-glutamate (4.9 mM), disodium fumarate (5.4 mM), sodium pyruvate (4.9 mM) and D-glucose (11.5 mM) and continuously bubbled with O_2/CO_2 (95%/5%). The jejunum was perfused with ice-cold buffer, after which a cold glass rod, pre-moistened with buffer, was inserted along the length of the intestine to stabilize the tissue. The preparation was continuously bathed in carbogenated Krebs buffer while the outer serosal and muscularis layers were gently stripped away, following scoring of the tissue along the mesenteric border with the blunt edge of a scalpel blade. Individual segments were cut from the jejunum and mounted in modified Ussing chambers (Harvard Apparatus Inc, Holliston, MA). Tissues were bathed in oxygenated Krebs buffer and maintained at 37°C. The exposed tissue surface area was 1.78 cm^2. After mounting, tissues were allowed 30 min to equilibrate before radiolabelled markers were added to the donor chamber. For the determination of mannitol and diazepam permeability, trace quantities of marker (approx. 0.5 μCi/chamber) were added to the donor chamber at time 0. In the experiments determining the permeability of digoxin, unlabelled drug (Lanoxin) was added to the donor and receiver chambers at a final concentration of 1 μM to inhibit non-specific adsorption. Approximately 1 μCi of labelled digoxin was then added to the donor chambers as a tracer. Since the mucosal and serosal buffers contained 11.5 mM glucose, glucose permeability was determined at this concentration. Approximately 0.5 μCi radiolabelled glucose was added to donor chambers to enable quantification of the flux. For all markers, samples (200 μL) were taken from the receiver chamber every 20 min for 3 h and were replaced with an equal volume of fresh supplemented Krebs buffer. Samples (200 μL) were also taken from the donor chamber at the start and end of the experiment, but these volumes were not replaced. Samples were added to 1.5 mL of scintillant cocktail (Starscint; Canberra-Packard, Meriden, CT) and radioactivity was counted using a liquid scintillation analyser (TriCarb 2000CA; Canberra-Packard). The P_{app} values, in cm s^{-1}, were calculated at steady state (which generally occurred over the period of 40–160 min) using the following equation:

$$P_{app} = dQ/dt \times (1/(A \times C_0)) \quad (1)$$

where dQ/dt represents the appearance rate of radiolabelled marker in the receiver chamber, A is the exposed surface area of the tissue and C_0 is the initial concentration in the donor chamber.

Electrical measurements

Electrical parameters were measured during the permeability experiments using two pairs of calomel Ag/AgCl electrodes bathed in 3 M KCl (Physiological Instruments Inc, San Diego, CA). One pair of electrodes was placed adjacent to each side of the tissue surface and used to measure the spontaneous transepithelial potential difference (PD). The other electrodes were placed at the distal end of each side of the diffusion chambers (to allow for equal current spread at the tissue surface) and used for passing current from an external six-channel (one channel for each diffusion chamber) voltage/current clamp (Physiologic Instruments Inc). Tissue segments with a PD of < 2 mV after the equilibration period, but before marker addition, were excluded from subsequent permeability experiments. The short-circuit current (Isc) required to reduce the spontaneous potential difference across the tissue preparation was measured when the voltage was clamped to zero. The mean Isc was calculated during periods of

steady-state flux (generally 40–160 min after marker addition).

BBM isolation and purification

BBMs were isolated from 10-cm sections of jejunum immediately distal to the segment used for permeability studies. The intestine was washed with ice-cold saline and blotted dry with lint-free paper before mucosal scrapings were gently taken using the blunt edge of a scalpel blade. Mucosal scrapings were transferred to pre-weighed tubes containing 5 mL mannitol–Tris buffer 1 (50 mM mannitol, 2 mM Tris HCl, pH 7.1) and stored at −20°C. BBMs were isolated as previously described (Kessler et al 1978). Briefly, the mucosal scrapings were thawed and homogenized in 10 mL of mannitol–Tris buffer 2 (50 mM mannitol, 10 mM Tris HCl, pH 7.1) using a polytron homogenizer (PT 1200C Kinematica AG Lihau, Switzerland) for 1 min at maximum speed (20 000 rev min^{-1}). $MgCl_2$ was added to obtain a final concentration of 10 mM, and the homogenate was gently mixed for 10 min and allowed to stand for 20 min. The homogenate was subsequently centrifuged at 3000 *g* for 10 min at 4°C. The supernatant was aspirated and centrifuged at 27 000 *g* for 30 min at 4°C. The second supernatant was removed, the pellet was resuspended in mannitol–Tris buffer 2 and centrifuged again at 27 000 *g* for 30 min at 4°C. The third supernatant was removed and the pellet was resuspended in 1 mL mannitol–Tris buffer 2. Within each dietary group, BBM preparations obtained from 4–5 animals were pooled and the proportional fatty acid content determined in duplicate as described below.

Fatty acid analysis of diets and BBMs

Fatty acid analysis of the BBM phospholipids was performed as previously described (Mann et al 1997). Briefly, lipids were extracted from the BBM pellet using chloroform/methanol (2:1, v/v) as adapted from the method of Folch et al (1957). Total phospholipids were separated by thin-layer chromatography on silica gel with a mixture of light petroleum, diethyl ether, acetic acid, and methanol (160:40:4:4, v/v). The phospholipids were saponified with 0.68 M KOH in methanol and fatty acid methyl esters (FAME) were prepared by methyl esterification with 20% BF_3 in methanol and then purified by passing through a silica Sep-Pak (Mann et al 1997). FAME were separated by capillary gas chromatography using a 50 m × 0.32 mm i.d BPX70, WCOT fused-silica capillary column (SGE, Melbourne, Australia) and analysed using a flame ionization detector. The column temperature was maintained at 95°C for the first 3 min, then increased at 10°C min^{-1} to 190°C, with helium as the carrier gas at a flow rate of 43 cm s^{-1}. Individual fatty acid peaks were identified by comparing the retention time with a known standard mixture of fatty acids (Mann et al 1997). The response factors for standard fatty acid methyl esters with carbon chain lengths from 14 to 22 and 0–6 double bonds were determined and used in the analysis of unknown samples. The proportional contribution of each fatty acid to the total fatty acid content was calculated by comparison of individual peak areas with the total peak area for all fatty acids in each chromatogram. The fatty acid content of the diets was similarly determined, although in this case the phosopholipid isolation step was not required.

Statistical analysis

Analysis of variance was used to confirm that there were no significant differences in the P_{app} values between rats within each dietary group. Permeability data within a dietary group were therefore pooled and are reported as the mean ± s.e.m. of a minimum of nine intestinal segments obtained from a minimum of three animals per dietary group. The P_{app} of marker compounds was compared between the five dietary treatments by one-way analysis of variance with a Bonferroni correction for multiple comparisons testing for significance at $P = 0.05$ (Minitab 12.1 Inc., State College, PA).

Results and Discussion

Animal characteristics

As shown in Table 1, there was no difference in the average weekly food intake or weight gain over the 30-day feeding period for rats fed the control and experimental diets. Rats fed the diet supplemented with SFA (butter) had a lower average weekly stool output compared with the other dietary groups. The reason for this anomaly is not known as the SFA-fed rats had normal food intake and weight gain over the dietary period.

BBM phospholipid fatty acid composition

The lipid-supplemented diets contained 18.4% (w/w) lipid, which is less than the fat content of a typical western diet (35–40% of dietary energy), but was chosen

Table 1 The characteristics of animals fed control (chow) and lipid-supplemented diets.

	Control (Chow; n = 9)	SFA (Butter; n = 9)	MUFA (Olive oil; n = 12)	ω-3 PUFA (Fish oil; n = 12)	ω-6 PUFA (Safflower oil; n = 9)
Food consumed (g/week)	153±14	171±14	173±19	151±11	198±55
Stool output (g/week)	17±3	8±2*	14±4	14±4	14±1
Weight gain (g)[a]	133±36	132±28	128±20	124±25	134±22
Final weight (g)	316±34	327±41	298±33	282±39	312±28

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; ω-3 and ω-6 PUFA, ω-3 and ω-6 polyunsaturated fatty acids, respectively. [a]Weight gain over dietary period (30±1 days). Data are means±s.e.m. *$P < 0.05$ compared with other lipid diets.

Table 2 Fatty acid composition of the lipid-supplemented diets.

	Proportion of total fatty acids (%)				
	Control (Chow)	SFA (Butter)	MUFA (Olive oil)	ω-3 PUFA (Fish oil)	ω-6 PUFA (Safflower oil)
14:0	0.97	8.31	0.10	6.53	0.17
16:0	15.1	30.3	10.7	17.3	6.26
16:1 ω-7	1.19	1.68	0.70	8.69	0.09
18:0	5.66	9.36	2.77	3.78	2.26
18:1 ω-9	36.4	44.0	73.4	9.43	14.6
18:2 ω-6	29.5	4.29	8.9	2.35	75.3
20:4 ω-6	ND	0.08	ND	1.12	ND
20:5 ω-3	0.58	0.11	ND	20.9	ND
22:5 ω-3	ND	0.13	ND	2.11	ND
22:6 ω-3	0.54	ND	ND	15.4	ND
Other[a]	10.1	1.8	3.44	12.4	1.29
Saturated[a]	22.2	48.4	14.0	32.0	9.04
Monounsaturated[a]	41.1	45.8	76.4	23.1	14.9
ω-3 Polyunsaturated[a]	7.06	1.19	0.77	40.7	0.52
ω-6 Polyunsaturated[a]	29.7	4.53	8.9	3.93	75.5

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; ω-3 and ω-6 PUFA, ω-3 and ω-6 polyunsaturated fatty acids, respectively. [a]A wider analytical profile was generated including other fatty acids present at lower concentrations (18:1 ω7; 18:3 ω3; 20:0; 20:1 ω9; 20:2 ω9; 20:3 ω6; 22:4 ω6 and 22:5 ω6). These have been grouped in the table under "other" for clarity. The wider profile was used to generate the summary data. ND, not detected.

to ensure tolerance and consumption of the diets by the animals. The fatty acid composition of the lipids used to make up the diets is shown in Table 2. The corresponding fatty acid composition of the BBM phospholipids at the end of the 30-day feeding period is shown in Table 3. The fatty acid content of the BBM phospholipids reflected the dietary intake of fatty acids (in particular the unsaturated fatty acids) and was similar to that reported previously (Brasitus et al 1985, 1989; Keelan et al 1990). The BBM phospholipids isolated from all the dietary groups had a similar content of 16:0 and 18:0 saturated fatty acids, which constituted approximately 20 and 30%, respectively, of the total fatty acid content of the BBM. The similarity in saturated fatty acid content of the BBM (Table 3) after pre-feeding diets with substantial differences in saturated fatty acid content (Table 2) highlights the likely presence of homeostatic mechanisms that control the extent of incorporation of saturated fatty acids into membranes. As expected, the BBM phospholipids in rats fed the MUFA diet (olive oil) had

Table 3 Fatty acid composition of jejunum brush border membrane phospholipids isolated from rats fed chow and lipid-supplemented diets.

	Proportion of total phospholipid fatty acids (%)				
	Control (Chow)	SFA (Butter)	MUFA (Olive oil)	ω-3 PUFA (Fish oil)	ω-6 PUFA (Safflower oil)
14:0	0.25	0.34	0.17	0.51	0.12
16:0	25.5	20.3	22.1	22.2	19.9
16:1 ω-7	0.47	0.74	0.19	1.06	0.11
18:0	29.8	30.4	29.0	28.0	29.7
18:1 ω-9	8.36	10.0	13.1	6.83	9.34
18:2 ω-6	11.5	13.0	9.44	7.2	15.0
20:4 ω-6	13.6	15.8	18.8	14.6	20.1
20:5 ω-3	0.22	1.2	0.12	5.88	0.09
22:5 ω-3	0.58	0.62	0.22	1.45	0.28
22:6 ω-3	1.57	2.40	1.4	7.13	1.03
Other[a]	8.18	5.28	5.52	5.12	4.33
Saturated[a]	56.6	51.6	52.2	51.6	50.4
Monounsaturated[a]	13.3	13.2	15.6	10.7	11.1
ω-3 polyunsaturated[a]	2.97	4.61	2.27	15.3	1.78
ω-6 polyunsaturated[a]	26.6	30.1	29.7	22.4	36.4

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; ω-3 and ω-6 PUFA, ω-3 and ω-6 polyunsaturated fatty acids, respectively. Intestinal segments (n = 4–5) from each dietary group were pooled and the brush border membrane (BBM) isolated. Results represent the mean of duplicate analyses of the pooled BBM preparation from each diet. [a]A wider analytical profile was generated including other fatty acids present at lower concentrations (18:1 ω7; 18:3 ω3; 20:0; 20:1 ω9; 20:2 ω9; 20:3 ω6; 22:ω6 and 22:5 ω6). These have been grouped in the table under "other" for clarity. The wider profile was used to generate the summary data. ND, not detected.

an elevated 18:1 ω-9 content compared with the other diets. As noted in previous studies (Garg et al 1992), supplementing the diet with ω-6 PUFA (safflower oil) was associated with an elevated 18:2 ω-6 and 20:4 ω-6 content in the BBM phospholipids. Similarly, feeding rats a ω-3 PUFA diet (fish oil) was shown to increase the long chain ω-3 fatty acids, 20:5 ω-3, 22:5 ω-3 and 22:6 ω-3, which was reflected in a markedly higher ω-3 to ω-6 ratio in the BBM phospholipids in this group, consistent with previous observations (Brasitus et al 1989).

The effect of dietary fatty acids on passive permeability

The paracelluar transport of compounds between enterocytes of the intestinal epithelium occurs via the tight junction complexes and typically represents a small contribution to the overall absorption of most drug molecules. Small, hydrophilic compounds such as mannitol, which exhibit minimal transcellular diffusion across the cell membrane, are often incorporated to monitor paracellular transport. In the current study, the P_{app} of mannitol across the intestinal segment was used as a marker of both intestinal tissue integrity and passive paracellular transport.

Permeability values for mannitol in the chow and different high-fat (18%, w/w) dietary groups are shown in Table 4. The transport of mannitol in the mucosal to serosal (m–s; absorptive) direction was not significantly different between the dietary groups ($P = 0.05$). For rats supplemented with dietary ω-3 and ω-6 PUFA, there was a trend (although not statistically significant) toward a decrease in the permeability of mannitol, suggesting that these fatty acids may lead to membrane changes that have the potential to alter tight junctional permeability. In this regard, it has been proposed that polyunsaturated fatty acids may modulate the cascade of events leading to the synthesis, structure and function of tight junctions (Jiang et al 1998). For example, in a human vascular endothelial cell line, the long chain fatty acids 18:3 ω-3, 18:3 ω-6 and 20:4 ω-6 were shown to up-regulate the expression of occludin, a key protein in the structure of tight junctions, and to result in a reduction in the paracellular permeability of a 10-kDa dextran (Jiang et al 1998). Although the data reported here did not reach statistical significance, the results are

Table 4 The mucosal to serosal apparent permeability (P_{app}) of the passive transport markers mannitol and diazepam.

	P_{app} ($\times 10^6$ cm s^{-1})				
	Control (Chow)	SFA (Butter)	MUFA (Olive oil)	ω-3 PUFA (Fish oil)	ω-6 PUFA (Safflower oil)
Mannitol	8.74±0.85	8.87±1.11	8.63±0.92	7.16±1.11	6.14±0.92
Diazepam	49.9±1.58	51.2±2.41	46.6±2.40	39.7±2.56*	37.0±2.03†

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; ω-3 and ω-6 PUFA, ω-3 and ω-6 polyunsaturated fatty acids, respectively. Data are mean±s.e.m. of a minimum of nine intestinal segments from at least three animals in each dietary group. *$P < 0.05$ for ω-3 PUFA compared with control and SFA. †$P < 0.05$ for ω-6 PUFA compared with control, SFA and MUFA.

consistent with these previous trends. It is possible that the permeability of larger markers, such as dextrans, may have been more discriminating in assessing subtle changes in paracellular transport when compared with the much smaller paracellular probe (mannitol) used in the current study.

Since the surface area of the enterocyte is much larger than the cross-sectional area of the paracellular channels, transcellular diffusion across the enterocyte is quantitatively the predominant route of absorption for most drug molecules. In these studies, the moderately lipophilic benzodiazepine, diazepam (log P = 2.8), was used as a marker of passive transcellular transport across the excised tissue segments. The permeability of diazepam in animals pre-fed diets containing 18% (w/w) SFA (butter) and MUFA (olive oil) was similar to that for the animals fed the chow diet (containing 5% fat) as shown in Table 4. Feeding rats diets enriched with either ω-3 or ω-6 PUFA led to an approximate 20% decrease ($P < 0.05$) in the P_{app} of diazepam across the jejunum when compared with the control and SFA-fed animals. These results are consistent with previous studies in which rats fed diets supplemented with fish oil (20:5 ω-3, 22:5 ω-3 and 22:6 ω-3) exhibited reduced uptake of lipophilic nutrients such as fatty acids and cholesterol into isolated intestinal segments when compared with rats fed diets rich in SFA (16:0 and 18:0) (Thomson et al 1986; Churnratanakul et al 1990). Although the mechanism for these apparent changes is unknown, the authors suggested a role for altered resistance of the unstirred water layer (Churnratanakul et al 1990), or changes to membrane structure leading to changes in intrinsic membrane permeability (Thomson et al 1986). Alteration to the fatty acid content of the BBM phospholipids has previously been shown to lead to altered packing of membrane phospholipid moieties and variation in membrane fluidity (Keelan et al 1990; Proulx 1991). For example, in rats fed a diet containing 20% fish oil, the rotational rate of the fluorescent anisotropy probe DPH (1,6-dipehyl-1,3,5-hexatriene) in the BBM was reduced when compared with rats fed a butter-supplemented diet, suggesting an increase in the packing order of the BBM and a reduction in membrane fluidity (Brasitus et al 1989). In contrast, other studies in rats fed diets supplemented with fish oil and a control diet have either failed to show a difference in measures of BBM fluidity (even in the presence of altered membrane enzyme activity) (Stenson et al 1989), or have described an increase in fluidity (Brasitus et al 1985). These apparently conflicting results suggest an incomplete understanding of the complex relationship between membrane structure, fluidity and permeability. Notwithstanding these inconsistencies, the data described here indicate that diets rich in PUFA may lead to a small reduction in transcellular permeability, at least for low molecular weight, relatively lipophilic probes such as diazepam.

The effect of dietary fatty acids on markers of efflux pathways and active transport

P-glycoprotein (P-gp) is an ATP-dependent efflux transporter expressed in the apical BBM of epithelial cells in the small intestine, and has recently been recognized as a potentially important limiting factor in the oral bioavailability of several structurally diverse compounds (Wacher et al 1995; Watkins 1997). Digoxin, a cardiac glycoside, is a substrate for intestinal P-gp (Cavet et al 1996) and was used in the present study as a marker to determine the potential effects of dietary lipids containing different fatty acids on this efflux pathway. In chow-fed animals, the serosal to mucosal flux (s–m) of digoxin, which represents the sum of both active efflux

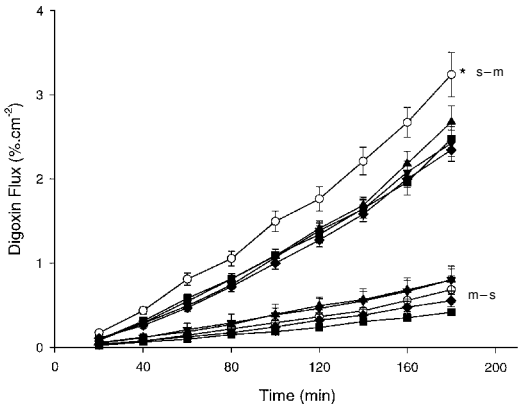

Figure 1 The effect of feeding diets of different fatty acid composition on the flux of digoxin across rat jejunum. Results are expressed as the mean±s.e.m. of a minimum of nine intestinal segments from at least three animals in each dietary group: control (chow; ○), saturated fatty acids (SFA; ▲), monounsaturated fatty acids (MUFA; ◆), ω-3 polyunsaturated fatty acids (ω-3 PUFA; ■) and ω-6 polyunsaturated fatty acids (ω-6 PUFA; ▼). Flux was measured in the mucosal to serosal direction (m–s) and serosal to mucosal direction (s–m). The source of fatty acids for the respective diets was butter (SFA), olive oil (MUFA), fish oil (ω-3 PUFA) and safflower oil (ω-6 PUFA). $^*P < 0.05$.

and passive permeability, was higher than the m–s flux, consistent with P-gp-mediated efflux (Figure 1). The steady-state digoxin flux for chow-fed animals was 1.21% $cm^{-2}\,h^{-1}$ (s–m) and 0.16% $cm^{-2}\,h^{-1}$ (m–s). The s–m digoxin flux was reduced in tissues obtained from animals fed the high-fat diets when compared with chow-fed animals, although no lipid-specific trends were observed (Figure 1). The lack of specificity when compared with the specific changes observed in BBM lipid constituents suggests that the decrease in s–m flux may result generically from the higher fat content of the experimental diets (18%, w/w) as opposed to any specific change in BBM lipid structure. The reduction in s–m digoxin flux seen in tissues obtained from animals pre-fed the SFA-supplemented diets (where the BBM lipid profile was not significantly different compared with control) further supports the limited role of specific changes to BBM lipids in this reduction in s–m digoxin permeability.

The reduction in s–m digoxin flux seen in the lipid-fed groups was not accompanied by a corresponding increase in the m–s flux, as would be expected in the event of typical P-gp inhibition. For example, in tissues obtained from animals pre-fed the diet supplemented with fish oil, the digoxin steady-state flux was 0.82% $cm^{-2}\,h^{-1}$ (s–m) and 0.1% $cm^{-2}\,h^{-1}$ (m–s). Manifestation of an attenuation in P-gp activity solely in s–m (and not m–s) transport has been reported previously for vinblastine, cimetidine and ranitidine (Hunter et al 1993; Collett et al 1999). In the case of these compounds, however, the asymmetry in inhibition was thought to reflect a substantial paracellular (and therefore P-gp independent) contribution to m–s transport, and a larger transcellular (and therefore P-gp dependent) s–m transport component (resulting from improved drug access to the enterocyte via the more permeable basolateral membrane). In the case of digoxin, Cavet et al (1996) described a classical inhibition profile in the presence of verapamil in Caco-2 cells (increased apical to basolateral transport and an equal and opposite decrease in basolateral to apical transport), whereas Fromm et al (1999) have shown a larger change in basolateral to apical digoxin flux in the presence of 5 μM quinidine. Because of inconsistencies in the P-gp inhibition profile of digoxin in the literature and the lack of data in excised tissues, we examined the intestinal permeability of digoxin in the presence of 100 μM verapamil (a competitive inhibitor of P-gp). Verapamil was added to both the mucosal and serosal chambers of permeability experiments conducted using intestinal tissue taken from chow-fed animals and animals fed fish oil (as a representative group of lipid-fed animals). In each group, inclusion of verapamil resulted in a significant decrease in the s–m flux of digoxin and an equal and opposite increase in the m–s flux. For example, in animals fed fish oil, the mean digoxin steady-state flux in the presence of 100 μM verapamil was 0.51% $cm^{-2}\,h^{-1}$ (s–m) and 0.45% $cm^{-2}\,h^{-1}$ (m–s). These results suggest that classical inhibition of P-gp (i.e. in the presence of verapamil) results in equal effects on both m–s and s–m flux of digoxin. The lipid dietary effects measured in the absence of verapamil (Figure 1) may therefore not reflect direct P-gp inhibition (since there was no measurable effect on m–s transport). Alternatively, the data may reflect a simple reduction in the permeability of digoxin across the BLM (as opposed to the BBM) and therefore reduced passive s–m transcellular transport and/or reduced access to the P-gp transporter for serosally applied digoxin.

The active transport of D-glucose was used to probe the potential impact of different dietary lipids on the activity of Na^+-dependent co-transport pathways. The apparent flux of D-glucose across excised intestinal tissue in the different dietary treatment groups is shown in Figure 2. Supplementing the diet with lipid was shown to moderately (but not significantly) enhance the m–s flux of D-glucose in rats fed the SFA, MUFA and ω-3 PUFA diets relative to the chow-fed rats. In contrast, enriching the diet with ω-6 PUFA (18:2 ω-6) was associated with a significant inhibition of D-glucose

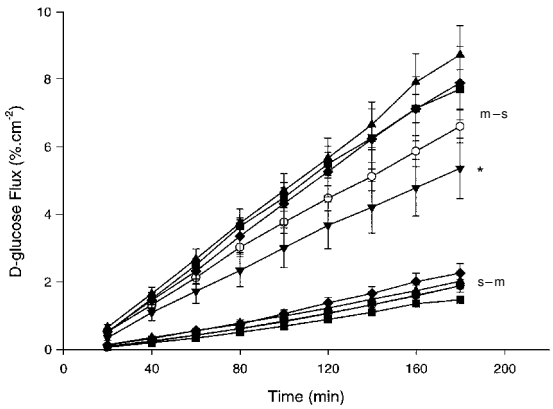

Figure 2 The effect of feeding diets of different fatty acid composition on the flux of D-glucose across rat jejunum. Results are expressed as the mean±s.e.m. of a minimum of nine intestinal segments from at least three animals in each dietary group: control (chow; ○), saturated fatty acids (SFA; ▲), monounsaturated fatty acids (MUFA; ◆), ω-3 polyunsaturated fatty acids (ω-3 PUFA; ■) and ω-6 polyunsaturated fatty acids (ω-6 PUFA; ▼). Flux was measured in the mucosal to serosal direction (m–s) and serosal to mucosal direction (s–m). The source of fatty acids for the respective diets was butter (SFA), olive oil (MUFA), fish oil (ω-3 PUFA) and safflower oil (ω-6 PUFA). *$P < 0.05$ for the ω-6 PUFA group significantly different compared with SFA, MUFA and ω-3 PUFA, but not from the control group.

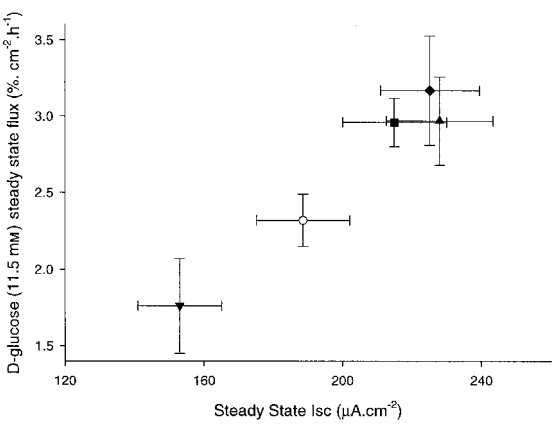

Figure 3 The relationship between short circuit current (Isc) and the steady state flux of D-glucose (11.5 mM) in rat jejunum ($r^2 = 0.96$). Results are expressed as the mean±s.e.m. of a minimum of nine intestinal segments from at least three animals in each dietary group: control (chow; ○), saturated fatty acids (SFA; ▲), monounsaturated fatty acids (MUFA; ◆), ω-3 polyunsaturated fatty acids (ω-3 PUFA; ■) and ω-6 polyunsaturated fatty acids (ω-6 PUFA; ▼). The source of fatty acids for the respective diets was butter (SFA), olive oil (MUFA), fish oil (ω-3 PUFA) and safflower oil (ω-6 PUFA).

transport in the m–s direction ($P < 0.05$). The inhibition of absorptive D-glucose flux in rats fed ω-6 PUFA was not reflected in a concomitant increase in the s–m flux. This was most likely owing to the predominance of paracellular transport when D-glucose is presented to the BLM. Limited transcellular transport in the s–m direction limits access of D-glucose to the SGLT1 transporter, which is located on the apical membrane (Thorens 1996). Consequently, D-glucose transport across the enterocyte in the s–m direction is likely to be relatively insensitive to changes in the activity of the apical membrane-resident SGLT1.

The results of this study are consistent with previous intestinal accumulation studies suggesting limited differences in D-glucose uptake in rats fed isocaloric diets enriched with SFA, MUFA or ω-3 PUFA at a D-glucose concentration of 11.5 mM (Thomson et al 1987b, 1988). These previous studies, however, did not include a control chow group for comparison. Perin et al (1997) examined glucose uptake into everted rat intestinal rings after pre-feeding both standard rat chow and semi-synthetic diets containing 20% fat (w/w), rich in either polyunsaturated (C18:2 ω-6) or saturated fat. Consistent with the data reported here, these studies showed that D-glucose uptake rates were highest after pre-feeding saturated fat, intermediate after the chow diet and significantly reduced after pre-feeding the polyunsaturated diet.

Studies in both control and diabetic animals (in which glucose uptake is enhanced) have also reported reduced glucose uptake after pre-feeding diets rich in PUFA (Thomson et al 1987b; Churnratanakul et al 1990). Direct comparisons, however, have generally not been made between ω-6 and ω-3 fatty acids and in many cases the inhibitory action of PUFA on glucose uptake has been attributed to ω-3 PUFA present in fish oil (Churnratanakul et al 1990) (as opposed to ω-6 PUFA in safflower oil). Others have also reported enhanced glucose uptake into rat intestinal sheets (as opposed to the reduced uptake reported here) after pre-feeding a high linoleic acid (C18:2 ω-6) diet when compared with a chow or saturated fatty acid diet (Thomson et al 1987a). An increase in glucose V_{max} as assessed by uptake into enterocyte BBM vesicles obtained from animals pre-fed menhaden (fish) oil as opposed to saturated fat (butter) has also been reported (Brasitus et al 1989). The reasons for the variability in observations between studies are not known, but likely reflect animal-to-animal differences and variation in the in-vitro preparations used (e.g. uptake into everted rings, tissue sheets or BBM vesicles versus permeability across intestinal segments).

The Na^+ concentration gradient that drives the active transport of D-glucose is maintained by the Na^+/K^+-ATPase situated on the BLM. In this regard, the Isc required to reduce the spontaneous transepithelial

potential across the enterocyte may be used as an indicator of the total ion flux across the epithelium and predominantly reflects the flux of Na^+ across the intestinal wall (Schultz & Zalusky 1964). Changes to the Isc, therefore, should reflect changes to Na^+-dependent transport processes such as that associated with the active transport of glucose. In line with this suggestion, the Isc measured during the steady-state period was found to be proportional to the m–s flux of D-glucose in the different dietary treatments as shown in Figure 3 ($r^2 = 0.96$, $P < 0.05$). The Isc at steady state was significantly lower in rats fed the ω-6 PUFA diet ($153.7 \pm 12.0\ \mu A\ cm^{-2}$; $P < 0.05$) compared with the other lipid-fed rats (SFA: $228.0 \pm 15.3\ \mu A\ cm^{-2}$; MUFA: $225.2 \pm 14.3\ \mu A\ cm^{-2}$; ω-3 PUFA: $215.4 \pm 15.0\ \mu A\ cm^{-2}$) and was lower (although not significantly) than the chow-fed rats ($188.6 \pm 13.4\ \mu A\ cm^{-2}$).

Conclusion

Altering the fatty acid content of different dietary lipids has been shown to modify the fatty acid composition of the BBM phospholipids in rat jejunum in line with previous investigations. The principal impact of the diets (which had vastly different proportions of saturated, monounsaturated and polyunsaturated fatty acids) on the BBM fatty acids was manifest in changes to the unsaturated fatty acid proportions in the BBM. No statistically significant differences were seen in the paracellular permeability of mannitol in animals fed different lipid diets, however a small decrease in the passive transcellular permeability of the lipophilic marker, diazepam, was detected in animals pre-fed diets rich in either ω-3 or ω-6 PUFA. Each of the lipid-rich diets led to a small (approx. 20%) decrease in the s–m flux of digoxin, but these results may have been manifest through changes in digoxin permeability across the BLM, rather than a specific inhibition of the P-gp efflux pump. In agreement with recent studies detailing changes to D-glucose uptake into intestinal tissue, the m–s flux of D-glucose was increased (although not to a statistically significant degree) after pre-feeding diets rich in SFA, MUFA and ω-3 PUFA, and significantly decreased ($P < 0.05$) after pre-feeding with ω-6 PUFA-rich diets. There was a direct correlation between the m–s flux of D-glucose in the different dietary groups and the steady-state Isc. The impact of these small changes in in-vitro permeability on the extent of in-vivo absorption remains to be determined, but may be important in relation to the dietary management of intestinal disorders such as inflammatory bowel disease and diabetes (Thomson et al 1996; Endres et al 1999) and the potential impact of these treatments on concomitant drug therapy.

References

Brasitus, T. A., Davidson, N. O., Schachter, D. (1985) Variations in dietary triacylglycerol saturation alter the lipid composition and fluidity of rat intestinal plasma membranes. *Biochim. Biophys. Acta* **812**: 460–472

Brasitus, T. A., Dudeja, P. K., Bolt, M. J., Sitrin, M. D., Baum, C. (1989) Dietary triacylglycerol modulates sodium-dependent D-glucose transport, fluidity and fatty acid composition of rat small intestinal brush-border membrane. *Biochim. Biophys. Acta* **979**: 177–186

Burke, A., Lichtenstein, G. R., Rombeau, J. L. (1997) Nutrition and ulcerative colitis. *Baillieres Clin. Gastroenterol.* **11**: 153–174

Cartwright-Shamoon, J. M., Dodge, J. A., McMaster, C. (1995) A complex biochemical modulation of intestinal ion transport in rats fed on high-fat diets. *J. Pediatr. Gastroenterol. Nutr.* **20**: 36–43

Cavet, M. E., West, M., Simmons, N. L. (1996) Transport and epithelial secretion of the cardiac glycoside, digoxin, by human intestinal epithelial (Caco-2) cells. *Br. J. Pharmacol.* **118**: 1389–1396

Churnratanakul, S., Kirdeikis, K. L., Murphy, G. K., Wirzba, B. J., Keelan, M., Rajotte, R. V., Clandinin, M. T., Thomson, A. B. (1990) Dietary omega-3 fatty acids partially correct the enhanced in vivo uptake of glucose in diabetic rats. *Diabetes Res.* **15**: 117–123

Collett, A., Higgs, N. B., Sims, E., Rowland, M., Warhurst, G. (1999) Modulation of the permeability of H_2 receptor antagonists cimetidine and ranitidine by P-glycoprotein in rat intestine and the human colonic cell line Caco-2. *J. Pharmacol. Exp. Ther.* **288**: 171–178

Endres, S., Lorenz, R., Loeschke, K. (1999) Lipid treatment of inflammatory bowel disease. *Curr. Opin. Clin. Nutr. Metab. Care* **2**: 117–120

Folch, J., Lees, M., Sloane-Stanley, G. H. (1957) A simple method for the isolation and purification of total lipids from animal tissue. *J. Biol. Chem.* **226**: 497–509

Fromm, M. F., Kim, R. B., Stein, C. M., Wilkinson, G. R., Roden, D. M. (1999) Inhibition of P-glycoprotein-mediated drug transport: a unifying mechanism to explain the interaction between digoxin and quinidine. *Circulation* **99**: 552–557

Garg, M. L., Keelan, M., Thomson, A. B., Clandinin, M. T. (1992) Desaturation of linoleic acid in the small bowel is increased by short-term fasting and by dietary content of linoleic acid. *Biochim. Biophys. Acta* **1126**: 17–25

Hunter, J., Hirst, B. H., Simmons, N. L. (1993) Drug absorption limited by P-glycoprotein-mediated secretory drug transport in human intestinal epithelial Caco-2 cell layers. *Pharm. Res.* **10**: 743–749

Jiang, W. G., Bryce, R. P., Horrobin, D. F., Mansel, R. E. (1998) Regulation of tight junction permeability and occludin expression by polyunsaturated fatty acids. *Biochem. Biophys. Res. Commun.* **244**: 414–420

Keelan, M., Wierzbicki, A., Clandinin, M. T., Walker, K., Thomson, A. B. (1990) Alterations in dietary fatty acid composition alter rat brush border membrane phospholipid fatty acid composition. *Diabetes Res.* **14**: 165–170

Kessler, M., Acuto, O., Storelli, C., Murer, H., Muller, M., Semenza, G. (1978) A modified procedure for the rapid preparation of

efficiently transporting vesicles from small intestinal brush border membranes. Their use in investigating some properties of D-glucose and choline transport systems. *Biochim. Biophys. Acta* **506**: 136–154

Lindley, K. J., Muller, D. P., Milla, P. J. (1995) Effects of dietary polyunsaturated fatty acids on small intestinal secretory and absorptive function: studies in rat jejunum in vitro. *Clin. Sci.* **88**: 219–224

Mann, N., Sinclair, A., Pille, M., Johnson, L., Warrick, G., Reder, E., Lorenz, R. (1997) The effect of short-term diets rich in fish, red meat, or white meat on thromboxane and prostacyclin synthesis in humans. *Lipids* **32**: 635–644

Miura, S., Tsuzuki, Y., Hokari, R., Ishii, H. (1998) Modulation of intestinal immune system by dietary fat intake: relevance to Crohn's disease. *J. Gastroenterol. Hepatol.* **13**: 1183–1190

National Health and Medical Research Council (NHMRC) (1992) *Dietary guidelines for Australians*. Commonwealth of Australia, Canberra

National Health and Medical Research Council (NHMRC) (1997) *Australian code of practice for the care and use of animals for scientific research*. Commonwealth of Australia, Canberra

National Research Council (Subcommittee on Laboratory Animal Nutrition) (1995) *Nutrient requirements of laboratory animals*. 4th edn. National Academy Press, Washington DC, pp 11–15

Perin, N., Keelan, M., Jarocka-Cyrta, E., Clandinin, M. T., Thomson, A. B. (1997) Ontogeny of intestinal adaptation in rats in response to isocaloric changes in dietary lipids. *Am. J. Physiol.* **273**: G713–G720

Proulx, P. (1991) Structure–function relationships in intestinal brush border membranes. *Biochim. Biophys. Acta* **1071**: 255–271

Rosella, O., Sinclair, A., Gibson, P. R. (2000) Polyunsaturated fatty acids reduce non-receptor-mediated transcellular permeation of protein across a model of intestinal epithelium in vitro. *J. Gastroenterol. Hepatol.* **15**: 626–631

Schultz, S. G., Zalusky, R. (1964) Ion transport in isolated rabbit ileum. *J. Gen. Physiol.* **47**: 567–584

Stenson, W. F., Seetharam, B., Talkad, V., Pickett, W., Dudeja, P., Brasitus, T. A. (1989) Effects of dietary fish oil supplementation on membrane fluidity and enzyme activity in rat small intestine. *Biochem. J.* **263**: 41–45

Thomson, A. B., Keelan, M., Clandinin, M. T., Walker, K. (1986) Dietary fat selectively alters transport properties of rat jejunum. *J. Clin. Invest.* **77**: 279–288

Thomson, A. B., Keelan, M., Clandinin, M. T., Walker, K. (1987a) A high linoleic acid diet diminishes enhanced intestinal uptake of sugars in diabetic rats. *Am. J. Physiol.* **252**: G262–G271

Thomson, A. B., Keelan, M., Garg, M., Clandinin, M. T. (1987b) Spectrum of effects of dietary long-chain fatty acids on rat intestinal glucose and lipid uptake. *Can. J. Physiol. Pharmacol.* **65**: 2459–2465

Thomson, A. B., Keelan, M., Garg, M., Clandinin, M. T. (1988) Dietary effects of omega 3-fatty acids on intestinal transport function. *Can. J. Physiol. Pharmacol.* **66**: 985–992

Thomson, A. B., Keelan, M., Wild, G. E. (1996) Nutrients and intestinal adaptation. *Clin. Invest. Med.* **19**: 331–345

Thorens, B. (1996) Glucose transporters in the regulation of intestinal, renal, and liver glucose fluxes. *Am. J. Physiol.* **270**: G541–G553

Ungell, A. L., Andreasson, A., Lundin, K., Utter, L. (1992) Effects of enzymatic inhibition and increased paracellular shunting on transport of vasopressin analogues in the rat. *J. Pharm. Sci.* **81**: 640–645

Wacher, V. J., Wu, C. Y., Benet, L. Z. (1995) Overlapping substrate specificities and tissue distribution of cytochrome P450 3A and P-glycoprotein: implications for drug delivery and activity in cancer chemotherapy. *Mol. Carcinog.* **13**: 129–134

Watkins, P. B. (1997) The barrier function of CYP3A4 and P-glycoprotein in the small bowel. *Adv. Drug Deliv. Rev.* **27**: 161–170

Wild, G. E., Turner, R., Chao, L. G. S., Faria, J., Keelan, M., Clandinin, M. T., Thomson, A. B. R. (1997) Dietary lipid modulation of Na^+/glucose co-transporter (SGLT1), Na^+/K^+ ATPase, and ornithine decarboxylase gene expression in the rat small intestine in diabetes mellitus. *J. Nutr. Biochem.* **8**: 673–680