Pharmaceutical Research, Vol. 23, No. 8, August 2006 (© 2006)
DOI: 10.1007/s11095-006-9021-6

Research Paper

An Acute and Coincident Increase in FABP Expression and Lymphatic Lipid and Drug Transport Occurs During Intestinal Infusion of Lipid-Based Drug Formulations to Rats

Natalie L. Trevaskis,[1] Chun Min Lo,[2] Li Yun Ma,[2] Patrick Tso,[2] Helen R. Irving,[3] Christopher J. H. Porter,[1,4] and William N. Charman[1]

Received December 9, 2005; accepted March 27, 2006

Purpose. To determine a) whether administration of lipid-based formulations can acutely up-regulate the intestinal expression of I-FABP and L-FABP and b) whether this occurs coincidentally with an increase in intestinal lymphatic lipid and drug transport.
Methods. The expression of I-FABP and L-FABP mRNA (using q-PCR) and protein (using immunohistochemistry and Western blotting) in enterocytes was compared with data describing transport of lipid and drug into intestinal lymph following infusion of a set of lipid-based formulations.
Results. Administration of relatively small amounts of oleic acid (5–20 mg/h) over a 5 h period to rats acutely up-regulated the expression, and altered the intracellular distribution of, I-FABP and L-FABP in the enterocytes of the small intestinal epithelia. The increase in expression of I-FABP and L-FABP correlated well with previous data describing the transport of lipid and drug into intestinal lymph following infusion of the same formulations.
Conclusion. The expression and intracellular distribution of I-FABP and L-FABP are acutely influenced by lipid infusion over a time period relevant to feeding or the administration of pharmaceutical lipidic formulations, and these changes occur coincidentally with increased drug transport into the lymphatics.

KEY WORDS: drug absorption; fatty acid binding protein (FABP); halofantrine; lipid absorption; lymph; lymphatic drug transport.

INTRODUCTION

Intestinal lipid absorption is a complex process involving numerous membrane and cytosolic transport proteins and enzymes in the absorptive cells of the small intestine (SI) (enterocytes) (1). Dietary triglyceride (TG) is rapidly digested to 2-monoglyceride and fatty acid (FA) in the upper SI and subsequently absorbed into the enterocyte either passively or actively via processes facilitated by membrane transport proteins. Active transport is thought to predominate at low luminal lipid concentrations, whereas under high lipid loads, the majority of lipid absorption occurs passively (2,3). Proteins implicated in FA transport across the enterocyte plasma membrane include CD36 and the FA transporter (FAT) (1), plasma membrane fatty acid binding protein ($FABP_{pm}$) (2,4,5), fatty acid transport protein (FATP4) (6), GP330 (7) and caveolin (1). Subsequent diffusion of these lipid digestion products through the enterocytic cytoplasm occurs by passive diffusion, however, this process is thought to be facilitated by cytosolic binding proteins, such as the cytosolic fatty acid binding proteins (FABP) (8–10).

Lipid digestion products gain access to the systemic circulation either directly via the portal vein, or via lymph as TG-rich lipoproteins (LP). Similarly, orally administered drugs can access the systemic circulation via either the portal blood or the mesenteric lymphatic system. Substantial intestinal lymphatic drug transport, however, only occurs for highly lipophilic drugs and it is typically a consequence of drug association with the lipid-rich LP (11). Therefore, intestinal lymphatic lipid and drug transport are closely related and it is possible that the factors which influence lipid absorption (such as levels of expression of cytosolic FABP) may also influence lymphatic drug transport.

[1] Department of Pharmaceutics, Victorian College of Pharmacy, Monash University, 381 Royal Parade, Parkville, Victoria 3052, Australia.
[2] Genome Research Institute, Department of Pathology, University of Cincinnati, Cincinnati, OH, USA.
[3] Department of Pharmaceutical Biology and Pharmacology, Victorian College of Pharmacy, Monash University, Parkville, Victoria, Australia.
[4] To whom correspondence should be addressed. (e–mail: chris.porter@vcp.monash.edu.au)

ABBREVIATIONS: ANOVA, analysis of variance; BS, bile salt; CV, coefficient of variation; FA, fatty acid; FABP, fatty acid binding protein; FFM, fentanyl, fluanisone and midazolam anaesthetic mixture; Hf, halofantrine; HPLC, high performance liquid chromatography; I-FABP, intestinal FABP; L-FABP, liver FABP; LP, lipoprotein; LPC, L-α-lysophosphatidylcholine; OA, oleic acid; PL, phospholipid; SER, smooth endoplasmic reticulum; SI, small intestine; TG, triglyceride.

Intestinal FABP and liver FABP (I-FABP and L-FABP, respectively) are purported to facilitate the intracellular diffusion of FA across the cytosol of the enterocyte (8,10), and indirectly therefore to also increase uptake into the enterocyte by promotion of sink conditions within the cell. For example, *in vitro* studies have demonstrated that I-FABP and L-FABP bind both saturated and polyunsaturated long chain FA with high avidity (12) and facilitate FA transport between phospholipid (PL) membranes (13). Moreover, the rate of FA uptake into HepG2 cells has been shown to display a dependence on the levels of intracellular L-FABP expression (14). *In vivo* studies have further demonstrated that both I-FABP and L-FABP are maximally expressed in the upper half of the small intestine (SI) where maximal lipid absorption occurs (1).

The expression of I-FABP and L-FABP in the SI is responsive to the lipid content of the diet. For example, up-regulation of I-FABP and L-FABP has been demonstrated in response to either a chronic (>7 day) high fat diet (9,15–17) or long term incubation of cultured cells with increased levels of FA (18). Acute *in vitro* up-regulation of I-FABP and L-FABP has also been demonstrated following a 2–8 h incubation of Caco-2 cells with increased levels of butyric acid, oleic acid (OA) or phosphatidylcholine (18) or following *in situ* incubation of mouse distal ileum with saturated and unsaturated long chain FA (but not medium chain FA) for 16 h (15).

Significantly, acute *in vivo* up-regulation of I-FABP or L-FABP in the SI during intestinal lipid absorption has not been reported. Therefore, the first aim of this study was to determine whether *in vivo* expression of I-FABP and L-FABP in the SI is up-regulated following intestinal lipid infusion over a time frame (e.g. 5 h) relevant to the absorption of lipid from a meal or pharmaceutical lipid-based formulation. A second aim was to determine whether these putative acute changes in the expression of I-FABP and L-FABP may be related to, or occur coincidentally with, the extent of lymphatic lipid and drug transport after intraduodenal infusion of lipid-based formulations.

MATERIALS AND METHODS

Composition and Preparation of Lipid-Based Formulations

An infusion rate of 5–20 mg/h OA in rats was chosen as it approximates, on a mg/kg basis, a human-relevant lipid dose of 1.2–4.7 g/h. L-α-lysophosphatidylcholine (LPC, 5.2 mg/h) was added to one of the formulations as this has previously been shown to enhance lymphatic lipid transport after intraduodenal administration (19–21). The infusion rate of bile salt (BS, sodium taurocholate) was 14 μmol/h which reflects the physiological input rate from rat bile under fasting conditions (22).

Lymphatic drug transport data from a previous study (23) are referred to in the current study. In brief, these data were collected after continuous intestinal infusion of essentially identical lipid-based formulations at 2.8 ml/h for 5 h, but where each 2.8 ml volume contained 100 μg halofantrine (Hf) (and 1 μCi of ^{14}C-OA where lymphatic FA transport rates were assessed). ^{14}C-OA and Hf were incorporated into the lipid phase (comprising either 5 mg OA, 20 mg OA or 20 mg OA/5.2 mg LPC) prior to dispersion in two different aqueous phases. The aqueous phases studied were either 0.2% (w/v) Tween 80 in normal saline (pH 7.0) or a bile salt (BS) solution comprising 5 mM sodium taurocholate in phosphate buffer (pH 6.9). Formulations were emulsified using an ultrasonicator and their stability assessed as previously described (24).

Surgical Procedures

All surgical and experimental procedures were conducted in accordance with the Australian and New Zealand Council for the Care of Animals in Research and Teaching guidelines and approved by the local institutional Animal Ethics Committee. Male Sprague–Dawley rats (280–320 g) were fasted overnight with free access to water. For Part 1 of the study, anaesthesia was initiated using a 3 ml/kg intraperitoneal (IP) injection of 'FFM' containing one part Hypnorm® (0.2 mg/ml fentanyl/10 mg/ml fluanisone; Janssen), one part Hypnovel® (5 mg/ml midazolam; Roche) and two parts Milli-Q water and maintained as described previously (24). In Part 2 of the study, anaesthesia was initiated and maintained with two hourly intraperitoneal injections of 50 mg/kg sodium pentobarbitone (Fatal Plus®, Vortech Pharmaceuticals, MI).

A tracheal cannulation was performed using a 3 cm piece of polyethylene tubing (2 mm id, Clay Adams, NJ) to maintain a clear airway. The duodenum was cannulated 1 cm below the pylorus with a J-shaped heat-moulded segment of polyethylene tubing (PE 50, Clay Adams, NJ) secured with instant cyanoacrylate adhesive. Anaesthetised rats were maintained on a heated pad at 37°C (Part 1) or in a controlled chamber at 37°C (Part 2) to maintain body temperature. At the conclusion of the surgical procedures, an intraduodenal infusion of normal saline (2.8 ml/h) was administered during a 0.5 h recovery period and then initiation of dosing was facilitated by changing the infusion to the respective lipid-based formulation (2.8 ml/h). Formulations were infused for the subsequent 5 h period. Samples were collected at the conclusion of the infusion period and rats were euthanized with a lethal overdose of sodium pentobarbitone.

Experimental Design

Study Part 1

Formulations comprising either (A) normal saline as control, (B) 0.2% Tween 80 in normal saline, (C) 5 mM BS solution, (D) 5 mg OA dispersed in 5 mM BS solution, (E) 20 mg OA dispersed in 0.2% Tween 80, (F) 20 mg OA dispersed in 5 mM BS solution or (G) 20 mg OA/5.2 mg LPC dispersed in 5 mM BS solution were intraduodenally infused (at 2.8 ml/h) to fasted, anaesthetised rats. At 5 h after initiation of the dosing (a period which provides steady state lipid and drug transport in lymph (23)) the SI was removed and the expression of I-FABP and L-FABP mRNA determined using q-PCR. In a separate study designed to give additional information as to the timescale of upregulation, intestinal segments were removed 2 h after initiation of dosing of formulation F (20 mg OA dispersed in 5 mM bile salt).

RNA extraction and cDNA preparation. Following the 2 or 5 h infusion period, the entire SI was removed, cut into three equal segments (upper, middle and lower third) and opened lengthwise. Enterocytes were removed from each segment by scraping with a clean glass microscope slide and homogenised in a mortar and pestle. Total RNA was extracted from 30 mg of each segment homogenate using a RNeasy Mini Kit® (Qiagen Limited, Australia) in accord with the manufacturer's protocol and diluted to 100 μl. The concentration of total RNA extracted from each enterocyte sample, and the RNA purity, were determined spectrophotometrically (WPA Biowave S2100 Diode Array Spectrophotometer, Radiometer, Denmark) at 260/280 nm. cDNA was reverse transcribed from 2 μg RNA using an Omniscript® kit with random hexamers (Qiagen Limited, Australia) in accord with the manufacturer's protocol and diluted to 20 μl.

Real time quantitative PCR (q-PCR). q-PCR was conducted on a Rotorgene RT-3000 (Corbett Research, Australia) and the data analysed using Rotorgene V5 software. Quantification of I-FABP and L-FABP expression was performed relative to a housekeeping gene (18S ribosomal RNA (25)) and measured in multiplex (26). A Taqman assay-on-demand kit (Rn 00565061, fatty acid binding protein 1, FABP 2; Applied Biosystems, NJ) was used to measure I-FABP using q-PCR. A Taqman® MGB probe and forward and reverse primers were designed to measure L-FABP using q-PCR (Applied Biosystems, NJ). The sequences were: L-FABP probe: 5′-CCCAAAGCTGATATAAT-3′, forward primer: 5′-AAGTACCAAGTGCAGAGCCAAGA-3′ and reverse primer: 5′-GACAGAAGGGATAGCCCCTCAT-3′. The multiplex reaction mix for the I-FABP and L-FABP q-PCR assays contained the Taqman® Universal PCR master mix and human 18S rRNA kit® (Applied Biosystems, NJ) which is appropriate for measuring rat 18S rRNA house keeping gene expression levels.

For multiplex q-PCR of L-FABP and 18S rRNA, the reaction mix contained a 2.5 μl sample of cDNA, Taqman PCR master mix®, 18 μ*M* L-FABP forward primer, 18 μ*M* L-FABP reverse primer, 5 μ*M* L-FABP probe, human 18S RNA kit® and water in a final volume of 50 μl. For multiplex q-PCR of I-FABP and 18S rRNA, the reaction mixture contained 1 μl sample of cDNA, Taqman PCR master mix®, I-FABP 20 × Taqman assay-on-demand kit®, human 18S RNA kit® and water in a final volume of 20 μl. The cDNA was amplified for one cycle at 50°C for 2 min and one cycle at 95°C for 10 min followed by 50 cycles of 95°C for 30 s (denaturing) and 60°C for 1 min (annealing and extension). Data were acquired at 60°C during cycling.

To enhance the accuracy of I-FABP and L-FABP q-PCR measurements, each sample was analysed in triplicate. Additionally, to ensure measurements from different q-PCR runs were cross-comparable, a set of reference samples obtained from the upper, middle and lower SI of a uninfused, fasted rat were analysed in each I-FABP or L-FABP q-PCR run. Expression data for I-FABP and L-FABP mRNA in all SI samples from experimental formulations was then calculated relative to the set of reference samples. To ensure that possible RNA or DNA contamination of SI samples was not affecting q-PCR results, each q-PCR analysis contained non-template controls consisting of enterocyte RNA samples where reverse transcriptase was not added (such that no cDNA was produced) prior to the q-PCR step.

Validation studies and data analysis. For I-FABP and L-FABP q-PCR assays, standards for I-FABP or L-FABP and 18S rRNA were prepared by 1/5, 1/10, 1/20, 1/50 and 1/100 dilutions of reference samples obtained from a fasted rat which was infused with normal saline for 5 h. The standards were analysed under the relevant q-PCR experimental conditions and standard curves (log [cDNA dilution] vs Ct (threshold cycle)) for I-FABP or L-FABP and 18S rRNA (housekeeping gene) produced. The inter- and intra-day accuracy and precision of the I-FABP, L-FABP and 18S rRNA assays were determined from triplicate q-PCR standard curves performed on three separate days and were acceptable (< ± 15%). The replication efficiencies for the assays were 1.93 and 1.87 for L-FABP and 18S rRNA, respectively, and 1.81 and 1.94 for I-FABP and 18S rRNA, respectively.

The expression of I-FABP or L-FABP mRNA in each SI segment (upper, middle or lower) of rats which had been infused with control (normal saline) or experimental lipid formulation was determined relative to their respective expression in a reference sample obtained from the upper third of the SI of an un-infused (fasted) rat thereby enabling assessment of the pattern of expression along the length of the SI. The average relative expression of the I-FABP and L-FABP mRNA in the SI was calculated from the mean of the relative expression values obtained for each third of the SI. This gave an indication of the total quantity of I-FABP and L-FABP mRNA present in the SI following infusion of each formulation. Additionally, the expression of I-FABP or L-FABP mRNA in each SI segment (upper, middle and lower) of rats infused with control (normal saline) or experimental lipid formulation was calculated relative to their expression in the same third of the SI of a set of reference samples from a un-infused, fasted rat thereby enabling assessment of the effect of the different lipid formulations on expression in each intestinal segment.

Study Part 2

To confirm that changes in mRNA expression were also reflected in changes to protein expression, additional studies were undertaken to assess changes in I-FABP and L-FABP levels (determined using Western blotting and immunohistochemistry) after administration of a representative lipid formulation for 5 h (20 mg OA dispersed in 5 mM BS solution) when compared to administration of normal saline for 5 h. After 5 h of infusion, the SI was removed and the expression and intracellular distribution of I-FABP and L-FABP protein determined.

Western blot analysis. Anti-rat I-FABP and L-FABP IgG antibodies were kindly donated by Professor Friedhelm Schroeder (Texas A & M University, USA) (27,28). Following 5 h infusion of either the normal saline or 20 mg OA/5 mM BS solution formulations, the SI was removed and cut into eight equal segments (labelled one through eight from duodenum to ileum). Segments two, four and six were used for Western blot analysis of the upper, middle and lower SI segments, respectively. One hundred milligrams of the SI

segments were homogenized in 1 ml of sampling buffer (consisting of one Complete Mini-Protease Inhibitor Cocktail Tablet® (Roche, NJ) dissolved in 10 ml of 0.1 M phosphate buffer, pH 7.4). Total protein concentration in homogenised samples was determined using a modified Lowry method (29). Protein samples (4.5 μg) and a pre-stained protein standard ladder (Benchmark pre-stained protein ladder, Invitrogen, CA) were loaded onto a 15% SDS gel, separated by SDS-PAGE and subsequently electro-transferred to polyvinylidene difluoride membranes (PVDF Immunoblot membrane, BioRad labs, Hercules, CA). The membrane was allowed to dry, soaked for 30 min in 0.4% (v/v) Tween 80 in Tris buffer pH 7.6 (TBST), blocked for 30 min with 5% (w/v) milk fat solution, incubated for 1 h with anti-rat I-FABP or L-FABP IgG diluted 1:1,500 in 5% milk fat solution, rinsed twice (for 5 min) with 5% milk fat, rinsed once (5 min) with 1.25% milk fat, incubated for 45 min with secondary anti-rabbit IgG (Polyclonal Goat Anti-Rabbit Immunoglobulins/HRP, DakoCytomation, Glostrup, Denmark) diluted 1:4,000 with 1.25% milk fat, washed twice (5 min each) with 1.25% milk fat and finally washed (5 min) with TBST. Bands were detected using ECL Western blotting detection reagent (Amersham Biosciences, Buckinghampshire, UK). Comparison of the migration distance of the I-FABP and L-FABP bands detected by the Western blot, with the molecular weight markers on the original SDS-PAGE gel, confirmed that the detected bands ran between the 10 and 15 kDa markers. This is consistent with the molecular weights of I-FABP and L-FABP (15 and 14 kDa (9), respectively).

Immunohistochemistry analysis. After 5 h of intraduodenal infusion, each rat received a lethal dose of pentobarbitone (0.6 ml of 390 mg/ml solution) and was immediately perfused via the aorta with 200 ml of room temperature normal saline followed by 400 ml of 4% (w/v) paraformaldehyde prepared in 0.1 M phosphate buffer (pH 7.4) maintained at 4°C. The SI was then removed and 3 cm segments collected from the upper third (from 1 cm below the entry of the bile duct which was more than 1 cm below the entry site of the intraduodenal infusion), the middle third (from 1 cm below the ligament of Treitz) and the lower third (from 1 cm above the ileo-caecal junction) of the SI. The samples were then left overnight in 4% paraformaldehyde phosphate buffer solution and then dehydrated for 2–3 days in 30% (w/v) aqueous sucrose solution until the intestinal segments no longer floated.

SI segments were embedded (#1310 M1 embedding matrix for frozen sectioning, Lipshaw Inc, Pittsburgh, PA) and 10 μm sections cut (CM 30505, Leica, Bannockburn, IL) onto microscope slides. Sections were incubated for 30 min at room temperature with 0.3% (v/v) H_2O_2 in 0.01 M phosphate buffer pH 7.4 in normal saline (PBS), blocked for 40 min at room temperature with 0.3% (v/v) Triton X-100/10% (v/v) normal goat serum (NGS) in PBS, incubated overnight at room temperature with primary IgG (anti-rat I-FABP or L-FABP) diluted 1:150 with blocking solution and incubated for 2 h at room temperature with anti-rabbit secondary IgG (568 Rabbit IgG labelling kit, Molecular Probes, Eugene, OR) diluted 1:400 in PBS. Sections were washed three times before and between each of these steps for 5–10 min with PBS. Sections were subsequently examined using fluorescence microscopy or confocal fluorescence microscopy. No autofluorescence was observed in control sections.

Fluorescent microscopy was conducted on an Olympus Bx 61 microscope and QI Camera (Olympus, Melville, NY) using Image Pro-plus software (Media Cybernetics Inc, Version 4.5.1.22). Images were analysed and FABP quantitated using Image J software (NIH, Bethesda, MD; http://rsb.info.nih.gov/ij/Java1.3.1_03). The amount of I-FABP and L-FABP in the upper, middle and lower SI of rats infused with control ($n = 2$) or lipid formulation ($n = 2$) was quantified from six regions of the fluorescent microscopic images of two slides for each rat.

Confocal fluorescent microscopy images were captured using an Axioplan 2® microscope, Axiocam HR® camera and Axiovision LE® software (Carl Zeiss, Thornwood, NY). The intracellular distribution of FABP was determined in the x, y and z direction from multiple z-stack images (captured using the confocal microscopy system) of two slides from each of the upper, middle and lower SI segments of rats infused with control ($n = 2$) or lipid formulation ($n = 2$) thereby confirming the intracellular location of FABP in three dimensions.

Statistical Analysis

Statistically significant differences were determined by ANOVA followed by Tukey's test for multiple comparisons at a significance level of $\alpha = 0.05$ using SPSS for Windows Version 11.5.0 (SPSS Inc, Chicago, Il).

RESULTS

Study Part 1: Expression of I-FABP and L-FABP mRNA in the SI Following Lipid Infusion

The expression of I-FABP and L-FABP mRNA in the upper, middle and lower regional segments of the SI, after infusion of either normal saline control or lipid formulations, was determined using q-PCR and expression was compared relative to a reference sample taken from the upper SI of an un-infused (fasted) rat. In the normal saline control group, the pattern of expression of I-FABP mRNA along the length of the SI (mean ± SEM, $n = 4$) (where the expression in each section of the SI is expressed relative to a reference sample obtained from the upper SI of a fasted rat that did not receive any infused material) was 1.03 ± 0.03 (upper region), 0.31 ± 0.05 (middle region) and 0.45 ± 0.07 (lower region). Similarly the patterns of expression of L-FABP mRNA in the normal saline control group (mean ± SEM, $n = 4$) were 1.05 ± 0.03 (upper region), 0.22 ± 0.05 (middle region) and 0.21 ± 0.04 (lower region). These data indicate that infusion of normal saline did not alter expression levels of both I-FABP and L-FABP mRNA (since the relative expression in the upper region was the same in saline infused animals as it was in the reference sample from the same region) and that I-FABP and L-FABP mRNA were more abundantly expressed in the upper SI.

The average relative expression of I-FABP and L-FABP mRNA in the SI of rats infused with control (normal saline)

or the lipid formulations was calculated as the mean of the expression values obtained in each segment (upper, middle, lower thirds). Within each segment, expression was calculated relative to the expression value obtained in a reference sample from the upper SI of an un-infused fasted rat. Fig. 1 (Panels 1A and 1B) depicts the change in the average relative expression of I-FABP and L-FABP mRNA in the SI of rats following a 5 h intraduodenal infusion of different lipid formulations. In general, the average expression of I-FABP mRNA in the SI was significantly increased following a 5 h infusion of all lipid formulations (including the 5 mM BS solution) when compared to control rats which received normal saline alone ($\alpha = 0.05$) (Panel A), where no increases to expression were seen. Infusion of the (lipid free) 0.2% Tween 80 formulation also did not alter the expression of I-FABP, suggesting that changes to the expression of I-FABP following infusion of 20 mg OA in 0.2% Tween 80 were mediated by the lipid present in the formulation (and not the surfactant). More specifically, the average expression of I-FABP mRNA in the SI increased significantly on infusion of BS, on infusion of increasing quantities of lipid (i.e. from 5 to 20 mg OA) or after addition of LPC to the 20 mg OA/5 mM BS solution ($\alpha = 0.05$). Panel 1B depicts the average expression of L-FABP mRNA in the SI which also increased

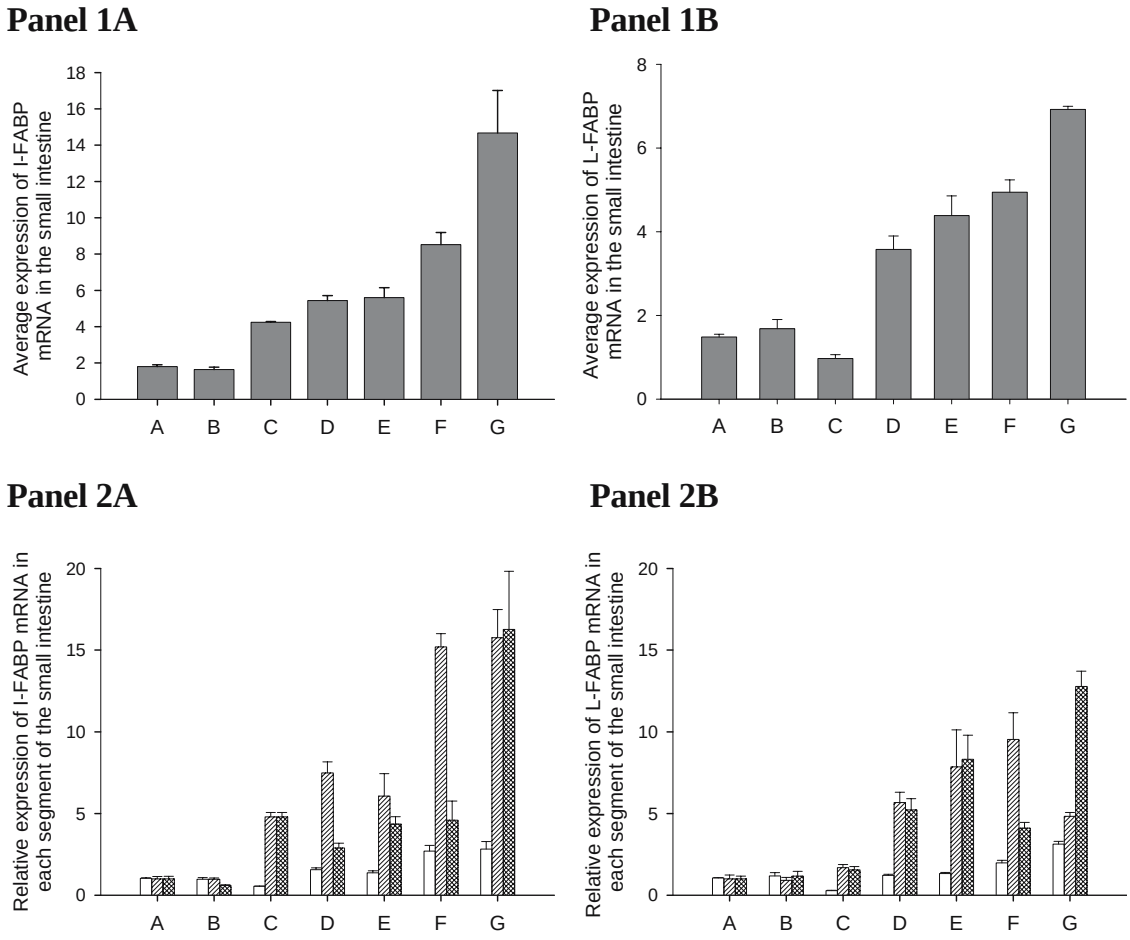

Fig. 1. *Panel* 1. The average relative expression (mean ± SEM, $n = 4$) of (A) I-FABP mRNA, and (B) L-FABP mRNA in the small intestine (*SI*) of rats following a continuous 5 h intraduodenal infusion of formulations comprising: A. normal saline control; B. 0.2 % Tween 80; C. 5 mM bile salt (*BS*) solution; D. 5 mg oleic acid (*OA*) dispersed in 5 mM BS solution; E. 20 mg OA dispersed in 0.2% Tween 80, F. 20 mg OA dispersed in 5 mM BS solution or G. 20 mg OA/5.2 mg lyso-phosphatidylcholine (LPC) dispersed in 5 mM BS solution per hour. I-FABP and L-FABP mRNA levels were measured using relative q-PCR. The relative expression of I-FABP and L-FABP mRNA in the *upper*, *middle* and *lower regions* of the SI was first calculated relative to the expression of I-FABP and L-FABP mRNA in the upper third region of untreated, fasted rats. The average relative expression of I-FABP and L-FABP mRNA in all thirds of the small intestine was then calculated from the mean of the relative expression values for all thirds. *Panel* 2. Regional small intestinal expression (mean ± SEM, $n = 4$) of (A) I-FABP mRNA, and (B) L-FABP mRNA in the upper (*open bars*), middle (*striped bars*) and lower (*cross-hatched bars*) region of rats following a continuous 5 h intraduodenal infusion of formulations comprising: A. normal saline control; B. 0.2 % Tween 80; C. 5 mM bile salt (*BS*) solution; D. 5 mg oleic acid (*OA*) dispersed in 5 mM BS solution; E. 20 mg OA dispersed in 0.2% Tween 80, F. 20 mg OA dispersed in 5 mM BS solution or G. 20 mg OA/5.2 mg lyso-phosphatidylcholine (*LPC*) dispersed in 5 mM BS solution per hour. I-FABP and L-FABP mRNA levels were measured using relative q-PCR and their expression in the *upper*, *middle* and *lower third region* of the SI were measured relative to the expression of I-FABP or L-FABP mRNA in a reference set of samples obtained from the same regions of untreated, fasted rats.

significantly after a 5 h infusion of all OA containing lipid formulations when compared to the data obtained after infusion of normal saline, although in this case no change was seen after administration of BS alone ($\alpha = 0.05$). A comparison of the data in Panels 1A and Panel 1B (Fig. 1) indicates that the increase in the average expression of I-FABP mRNA following infusion of the lipid formulations was greater than that observed for L-FABP mRNA.

To determine the region of the SI where expression of I-FABP and L-FABP changed most following intraduodenal infusion, expression levels in the upper, middle and lower regions of the SI were determined relative to the expression of I-FABP or L-FABP mRNA in the corresponding intestinal region of fasted (un-infused) rats. Fig. 1 (Panels 2A and 2B) depicts the expression of I-FABP and L-FABP mRNA, respectively, in each region of the SI following a 5 h infusion of control (normal saline) or lipid based formulations. For both I-FABP and L-FABP, infusion of 0.2% Tween 80 alone (formulation B) did not lead to any changes in regional expression levels of mRNA when compared with infusion of normal saline. In the lower 2/3 region of the SI (where levels of expression of both I-FABP and L-FABP mRNA in control animals infused with normal saline were lowest) infusion of all the lipid formulations increased the expression of both I-FABP and L-FABP mRNA significantly ($\alpha = 0.05$) when compared to expression levels in the respective segments of animals infused with normal saline for 5 h. More specifically, for I-FABP mRNA, expression levels in the middle 1/3 of the SI were increased more significantly ($\alpha = 0.05$) following infusion of formulations containing 20 mg OA and BS, in both the presence and absence of LPC (formulations F and G) when compared to infusion of BS alone (formulations C), 5 mg OA in BS (formulation D) and 20 mg OA in Tween 80 (formulation E). In the lower 1/3 of the SI expression levels of I-FABP mRNA were most significantly increased ($\alpha = 0.05$) following infusion of the 20 mg OA/5.2 mg LPC in BS formulation (formulation G) when compared to the other formulations (C, D, E and F). For L-FABP mRNA, the patterns were similar and expression in the middle 1/3 of the SI was significantly increased ($\alpha = 0.05$) for all the lipid containing formulations. Interestingly in the middle 1/3 of the intestine, addition of LPC (formulation G) did not increase expression levels above that seen after administration of 20 mg OA/BS (and in fact levels were reduced), whereas in the lower 1/3 of the intestine a significant increase in L-FABP mRNA expression levels was observed after administration of the LPC containing formulation (formulation G). In general, therefore it appears that infusion of OA leads to increases in the expression levels of both I-FABP and L-FABP mRNA levels, particularly in the middle 1/3 of the intestine, whereas the additional effects of addition of LPC are more evident (especially in the case of L-FABP) in the lower 1/3 of the SI.

In contrast, relatively small changes in FABP mRNA expression levels were evident in the upper 1/3 of the intestine (where endogenous levels of expression in control rats infused with normal saline were highest). Thus, only a small (albeit significant) increase in I-FABP mRNA expression levels were apparent in the upper region of the SI after administration of the 20 mg OA in BS solution formulation (with or without LPC) (i.e., formulations F and G (Panel 2A)). Similarly, a modest but significant increase in L-FABP expression was seen in the upper SI following infusion of the 20 mg OA/5.2 mg LPC in BS formulation (formulation G (Panel 2B)). Surprisingly, administration of 5 mM BS solution significantly decreased L-FABP mRNA expression in the upper third of the SI ($\alpha = 0.05$) to levels below that obtained in control (normal saline infused) animals.

To gain a better understanding of the timescale over which I-FABP and L-FABP mRNA were up-regulated in the small intestine, an additional group of rats were infused with the 20 mg OA/5 mM BS formulation for 2 h and the levels of I-FABP and L-FABP mRNA along the length of the SI measured using q-PCR and compared to the expression in control rats infused with normal saline alone. Over this timescale, the expression of I-FABP increased 1.5 ± 0.3, 2.1 ± 0.1 and 2.3 ± 0.2 fold and the expression of L-FABP 2.1 ± 0.1, 2.7 ± 0.1 and 2.2 ± 0.1 fold (mean $\pm$ SEM, $n = 4$ rats) in the upper, middle and lower thirds of the small intestine, respectively.

Overall, the expression of both I-FABP and L-FABP mRNA was particularly up-regulated in the lower 2/3 of the SI following infusion of the lipid formulations, and this was most evident in the data obtained after 5 h, although significant increases in expression levels (~2 fold) were also apparent after only 2 h. It should be noted, however, that although I-FABP and L-FABP exhibited the greatest overall increase in expression in the lower 2/3 region of the SI following experimental lipid infusion, the levels of I-FABP and L-FABP mRNA in the lower 2/3 were only increased to levels similar to those observed in the upper regions of the SI.

Study Part 2: Expression of I-FABP and L-FABP Protein in the SI Following Lipid Infusion

To examine whether the observed changes to expression levels of I-FABP and L-FABP mRNA were reflected in changes to protein expression, a further series of experiments were conducted where two groups of rats were infused with either normal saline or a representative lipid formulation (20 mg OA/5 mM BS solution) and I-FABP and L-FABP levels examined using either Western blot or immunohistochemical analysis.

Figure 2 (Panels A and B) depicts Western blots of I-FABP and L-FABP protein expression, respectively, in each region of the SI following a 5 h infusion of normal saline (control) or the 20 mg OA/5 mM BS solution formulation (i.e., formulation F). The data shown are representative data taken from single gels, however experiments were repeated in triplicate and similar patterns of up-regulation were observed in each case. The data for I-FABP are qualitatively consistent with the mRNA levels presented in Fig. 1 in that I-FABP is expressed most abundantly in the upper 2/3 of the SI in the fasted state and the expression of I-FABP increased relatively more in the lower 2/3 of the SI following lipid infusion. Also consistent with the mRNA levels presented in Fig. 1, L-FABP is expressed most abundantly in the upper 2/3 of the SI in the fasted state, is significantly up-regulated along the length of the intestine, and the region of most significant change is in the middle 1/3 of the SI.

The increase in the expression of I-FABP and L-FABP in each third of the SI was also estimated immunohistochemi-

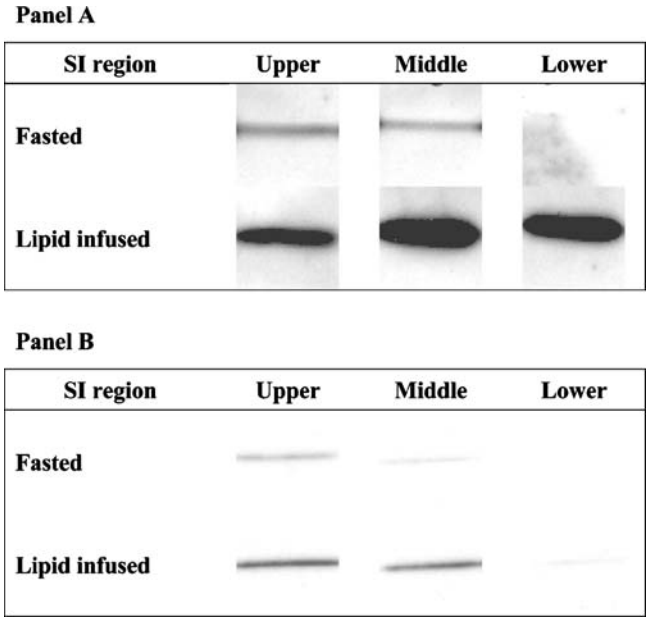

Fig. 2. Western blot quantitation of (A) I-FABP and (B) L-FABP protein expression in the *upper*, *middle* and *lower third region* of the small intestine (*SI*) of rats following continuous a 5 h continuous intraduodenal infusion with normal saline control or a formulation comprising 20 mg OA dispersed in 5 mM bile salt solution per hour. At the conclusion of the 5 h intraduodenal lipid infusion, SI segments were removed and total protein extracted from the mucosa and separated by SDS PAGE and blotted onto a PVDF membrane. Blots were probed with either anti-rat I-FABP IgG (1:1500) or anti-rat L-FABP IgG (1:1500) followed by a secondary anti-rabbit IgG (1:4,000). Each of the individual blots in Panel A and Panel B represent bands taken from a single gel. Background levels varied across gels (i.e., between panels A and B) but were consistent within a gel. Levels of both I-FABP and L-FABP in the *lower regions* of the SI in fasted animals were not detectable.

cally after infusion with either normal saline (control) or the 20 mg OA/5 mM BS solution formulation (i.e. formulation F). In the first series of experiments, sections were analysed by fluorescence microscopy (images not shown) and quantified using the pixel fluorescence intensity of the immunohistochemical images. The relative increase in I-FABP protein expression in each region of the SI following lipid formulation infusion compared with normal saline control (mean ± SEM, $n = 12$) was 3.82 ± 0.17 (upper region), 3.69 ± 0.18 (middle region) and 1.31 ± 0.03 (lower region). Similarly, the relative increase in L-FABP protein expression in each region of the SI was 2.23 ± 0.04 (upper region), 2.14 ± 0.08 (middle region) and 1.92 ± 0.02 (lower region). These data are reasonably consistent with the mRNA expression levels as quantified by q-PCR in Fig. 1, although the magnitude of the increase in protein expression determined immunohistochemically was less than the relative increases in mRNA expression determined by q-PCR, particularly in the lower 2/3 of the SI.

Sections were also examined using confocal fluorescence microscopy and Fig. 3 (Panels A and B) shows confocal immunohistochemistry images of the corresponding protein expression and intracellular distribution of I-FABP and L-FABP in enterocytes of either control rats or those infused with the 20 mg OA/5 mM BS solution formulation. In the fasted state, I-FABP was most clearly concentrated in the basolateral region of the enterocyte (although small quantities were also evident in close association with the apical membrane), whereas after lipid infusion, I-FABP was more abundantly and more widely distributed across the entire cytoplasm (three dimensional analysis, data not shown). In the fasted state, L-FABP was more diffusely located throughout the enterocyte cytoplasm when compared to I-FABP, although some evidence of concentration of L-FABP around the nuclear, apical and basolateral membranes was apparent in some enterocytes. Following lipid infusion, L-FABP was more concentrated in the cytoplasm as well as the nuclear, apical and basolateral membranes. The changes to FABP location were consistently observed across a series of x, y and z planes.

Intestinal Lymphatic Lipid and Drug Transport and I-FABP and L-FABP mRNA Expression in the SI Following Lipid Infusion

We have previously reported the rate of intestinal lymphatic lipid and drug transport following 5 h continuous intraduodenal infusion of the same lipid formulations as those employed in the current study, in mesenteric lymph duct cannulated rats (23). In these previous studies, the 5 h intraduodenal infusion of the lipid-based drug formulations induced a condition of steady state lipid and drug transport into intestinal lymph. Figure 4 compares the average expression of I-FABP and L-FABP mRNA in the SI determined in the current study with the previously described steady state rate of transport of lipid and drug into intestinal lymph. The average overall expression of I-FABP and L-FABP mRNA in the SI correlates well with the rate of lymphatic transport of total FA and Hf (% of dose per h) at steady state, particularly at higher lipid transport rates.

DISCUSSION

The intracellular lipid binding proteins I-FABP and L-FABP are believed to facilitate the cytosolic diffusion (and therefore indirectly the cellular uptake) of lipid digestion products during their intestinal absorption (10). Accordingly, the expression of I-FABP and L-FABP in the SI appears to be responsive to the lipid content of the diet. *In vivo* studies have demonstrated an up-regulation of I-FABP and L-FABP following chronic (days–weeks), long term increases in dietary fat consumption (9,15–18,30–32). The present study demonstrates, for the first time, that expression of both I-FABP and L-FABP in the SI increases *acutely* (i.e., within hours) during the process of intestinal lipid absorption. This study also shows that acute changes to the levels of expression of I-FABP and L-FABP in the SI occur coincidentally with changes to the extent of intestinal lymphatic transport of long chain lipids and drugs.

I-FABP and L-FABP mRNA and Protein Expression in the SI Following Lipid Infusion

In the fasted state, I-FABP and L-FABP were most abundantly expressed in the upper 2/3 of the SI. This data is consistent with previous studies in rats (6). In contrast, in mice, previous reports suggest that whilst L-FABP mRNA

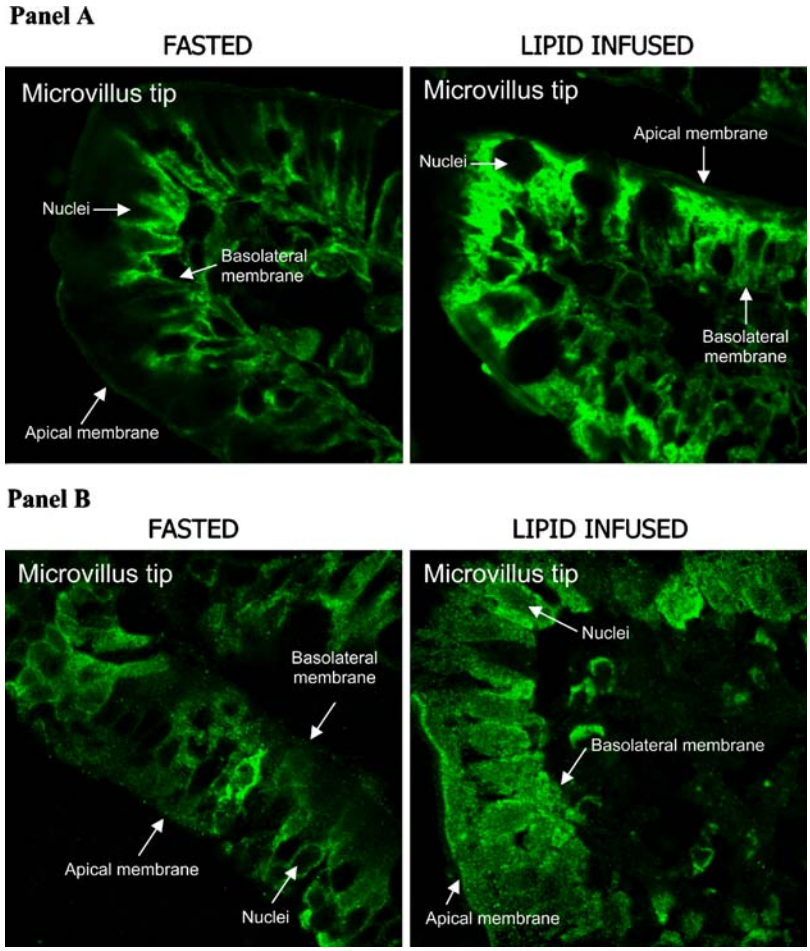

Fig. 3. Confocal immunohistochemical analysis of (A) I-FABP and (B) L-FABP location within enterocytes in the *middle third region* of the small intestine (*SI*) of rats following a continuous 5 h intraduodenal infusion with normal saline control or a formulation comprising 20 mg OA dispersed in 5 mM bile salt solution per hour.

levels are highest in the proximal jejunum, I-FABP mRNA levels are relatively low in the duodenum and increase progressively down the intestine to reach a higher level in the proximal ileum (15).

The average expression of both I-FABP and L-FABP mRNA and protein in the SI (Fig. 1, Panel 1 and Fig. 2) increased following a 5 h intestinal infusion of a relatively low lipid dose (5–20 mg/h) to rats. This is the first study to demonstrate that both I-FABP and L-FABP mRNA and protein expression may be acutely up-regulated, within a period of hours, during the time course of lipid administration thereby suggesting that the expression of I-FABP and L-FABP may potentially be controlled on a daily basis in response to dietary lipid intake.

I-FABP and L-FABP mRNA levels particularly increased in the lower 2/3 of the SI following lipid infusion. Again, the regional changes to FABP expression in the SI were similar to previous chronic studies where, for example, L-FABP and I-FABP levels increased more substantially in the ileum (1.4 and 1.5 fold, respectively) than the jejunum (1.3 and 1 fold, respectively) when rats were fed a high fat diet (containing 20–38% w/w fat) for 21–31 days compared to rats on a standard 5% w/w fat diet (9). That fasted levels of expression of I-FABP and L-FABP are normally high in the upper SI is consistent with the general understanding that the majority of lipid absorption occurs in the duodenum and jejunum (33). The current data, however, also suggests that the expression of I-FABP and L-FABP may be readily up-regulated in the lower regions of the SI during infusion of relatively low quantities of lipid, such that lipids which 'spill' over into the lower regions of the SI are efficiently absorbed.

Interestingly, infusion of BS alone increased expression of I-FABP in the lower 2/3 of the SI and decreased the expression of L-FABP in the upper reaches of the SI. The addition of BS or BS plus LPC to the 20 mg OA formulation also significantly increased the expression of I-FABP and L-FABP in the lower SI. Since BS and LPC are components of bile and were infused at a rate similar to the physiological input rate of bile into the rat intestine, these data suggest that bile may play a part in controlling the expression of FABP in the SI. Studies are ongoing to examine this possibility.

In the current study, a 5 h lipid infusion increased the average expression of I-FABP mRNA to a greater extent than that of L-FABP mRNA in the SI (Fig. 1 Panel 1). This is consistent with previous *in vitro* studies in caco-2 cells where the expression of I-FABP was upregulated to a greater extent than L-FABP after incubation in FA containing medium (18). In contrast, however, studies detailing changes to I-

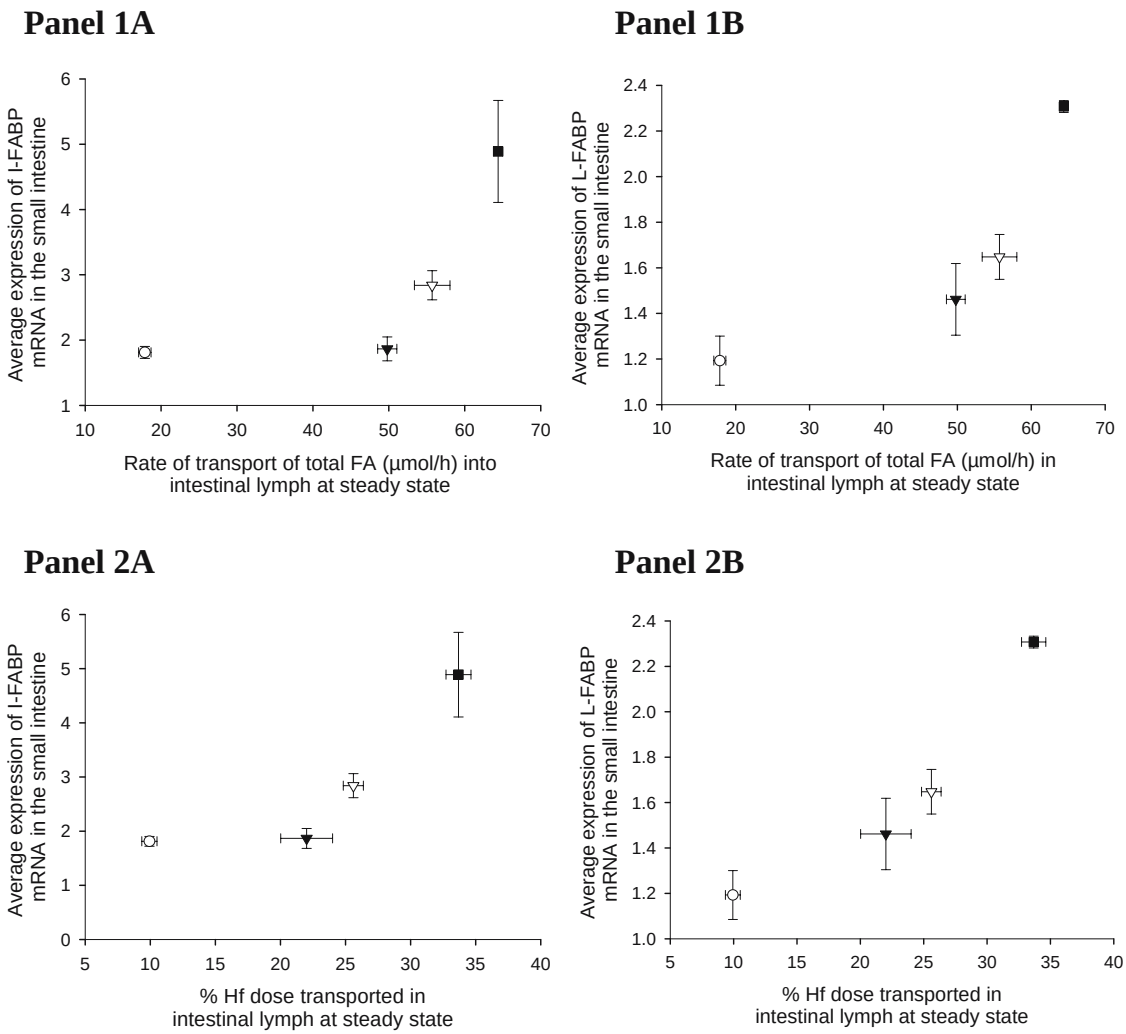

Fig. 4. The average expression (mean ± SEM, $n = 4$) of (A) I-FABP and (B) L-FABP mRNA in the small intestine of rats (calculated from the average of their expressions in the *upper*, *middle* and *lower regions* of the small intestine using q-PCR) *versus* the rate of total fatty acid (*FA*) transport (μmol/h) into intestinal lymph at steady state (*Panel* 1); or the percentage of the dose of halofantrine (*Hf*) transported into intestinal lymph at steady state in mesenteric lymph duct cannulated anaesthetised rats (*Panel* 2) following a 5 h continuous intraduodenal infusion of hourly lipid formulation doses containing 100 μg Hf dissolved in (a) 5 mg oleic acid (OA) dispersed in 5 mM bile salt (*BS*) solution (○), (b) 20 mg OA dispersed in 0.2% Tween 80 (▼), (c) 20 mg OA dispersed in 5 mM BS solution (▽) or (d) 20 mg OA/5.2 mg lyso-phosphatidylcholine dispersed in 5 mM BS solution (■).

FABP and L-FABP following chronic administration of a high fat diet have typically shown greater increases in the expression of L-FABP as opposed to I-FABP (15,18,31,32). In accordance with the apparently differential pattern of regulation of I-FABP and L-FABP they are also believed to have different roles in SI lipid absorption and processing (8). In particular, I-FABP is believed to be involved in TG re-synthesis and the formation of TG-rich lipoproteins (LP) in the enterocyte, whereas L-FABP appears to be closely associated with enterocyte-based PL synthesis (14,27,34–39).

The mechanisms by which the administered FA increased FABP expression was not addressed in the current study, however, available evidence suggests that FA stimulate FABP expression at the transcriptional level (15,40,41). Up-regulation is believed to occur through FA binding to a nuclear hormone receptor (NHR) followed by binding of the FA-NHR complex to the promoter region of the FABP gene to enhance FABP mRNA transcription (42). The genes that encode for both I-FABP and L-FABP display NHR binding sites (including peroxisome proliferator activated receptors (PPARs) and C/EBPα) in their promoter region (43). For example, FA can induce L-FABP expression in the SI via PPARδ activation (but not PPARα) and administration of PPARδ agonists induces L-FABP expression in the SI (30). In the current study, it is likely therefore, that infusion of FA induced an acute up-regulation of I-FABP and L-FABP expression via changes at the transcriptional level and possibly by binding to PPAR. Since the genes encoding other intestinal lipid transport and metabolic proteins such as FAT/CD36, FATP and Acyl CoA synthetases also possess PPAR binding sequences in their promoter region (42), it is further possible that they are also co-ordinately regulated and involved in an acute SI adaptation to lipid absorption. Further studies are required to confirm this possibility.

Intracellular Distribution of I-FABP and L-FABP within the Enterocyte Following Lipid Infusion

The intracellular distribution of I-FABP and L-FABP was also notably changed after administration of 20 mg/h of OA for 5 h (Fig. 2). Thus, I-FABP shifted from the basolateral (and to a lesser degree, apical) membranes in the fasted state, to relatively greater presence in the cytoplasm following lipid administration. Similarly, L-FABP was more abundantly expressed in the cytoplasm following lipid administration compared to the fasted state. These patterns of change are consistent with those previously reported (44) following administration of much larger lipid doses (2 ml bolus dose of corn oil, equivalent to 450–500 ml to a human on a mg/kg basis). The current studies therefore suggest that significantly lower, and more physiologically relevant amounts of lipid, are capable of inducing similar changes.

Intestinal Lymphatic Lipid and Drug Transport and I-FABP and L-FABP mRNA Expression in the Small Intestine During Lipid Infusion

Infusion of the series of lipid-drug formulations led to parallel changes in the average expression of I-FABP and L-FABP in the SI (current study) and the extent of drug transport into the lymph at steady state (data reproduced from (23)) (Fig. 4 (Panel 2)). The average expression patterns of I-FABP and L-FABP in the SI could also be correlated with the rate of transport of lipid into lymph (Fig. 4 (Panel 1)). The current data therefore raise the possibility that the levels of expression of I-FABP and L-FABP may influence the transport of both lipids and drugs into the intestinal lymphatics, and that a positive feedback mechanism may operate such that increases in lipid absorption stimulate upregulation of FABP which in turn promotes further increases in lymphatic lipid (and therefore drug) absorption.

The most likely mechanism by which increased levels of cytosolic FABP may enhance lymphatic drug transport is by facilitating lipid transport across the enterocyte and promoting lipoprotein synthesis, leading to an indirect increase in lymphatic drug transport occurring as a consequence of an increase in lymphatic lipid transport. However, recent studies have also shown that some drugs can bind FABP directly (45) raising the possibility that FABP may play a more direct role in intracellular drug distribution and potentially in the delivery of drugs to the site of lipoprotein assembly. Further studies, however, are required to confirm a causative link between FABP expression and lymphatic lipid and drug transport. In particular, the current data cannot differentiate between the possibility that the various lipid formulations differentially increased lymphatic lipid absorption via mechanisms independent of FABP expression, and that the increases in FABP expression occurred solely as a consequence of increased lipid absorption rather than the reverse (ie., that changes to FABP expression resulted in changes to lipid absorption). Furthermore, the changes reported here occurred over a 5 h period of constant infusion of the lipid formulations and only limited data are presented showing changes in mRNA levels at shorter time periods (2 h) after initiation of lipid infusions, and after infusion of varying quantities of lipid. Further studies are therefore required to confirm the time and lipid volume dependencies of the reported observations.

CONCLUSIONS

I-FABP and L-FABP are believed to facilitate the absorption and transport of lipid digestion products across the enterocyte. Previous studies have demonstrated transcriptional up-regulation of I-FABP and L-FABP following chronic ingestion of a high fat diet (9,15–18,30–32). The current study has, for the first time, demonstrated that I-FABP and L-FABP mRNA and protein expression levels in the SI are acutely up-regulated *in vivo* following infusion of relatively small lipid doses over relatively short time periods (2–5 h). Furthermore, the acute increase in the expression of I-FABP and L-FABP occurred coincidentally with increased rates of transport of lipids and drug into intestinal lymph. The data therefore raise the possibility that positive feedback mechanisms may acutely regulate lipid absorption at a transcriptional level, and thereby promote lymphatic drug transport. However, further studies are required to confirm whether a causative link exists between I-FABP and L-FABP expression levels and lymphatic lipid and drug transport.

ACKNOWLEDGMENTS

NLT gratefully acknowledges financial support provided by an Australian Postgraduate Scholarship. We thank Prof Colin Pouton (Monash University) for q-PCR advice and access to equipment, Professor Friedhelm Schroeder (Texas A & M University) for kindly donating anti-rat I-FABP and L-FABP antibodies, Amin Yu and Jildou Hoekstra (University of Cincinnati) for assistance with Western blotting and Adam Southon (University of Melbourne) for q-PCR assistance.

REFERENCES

1. B. K. Nordskog, C. T. Phan, D. F. Nutting, and P. Tso. An examination of the factors affecting intestinal lymphatic transport of dietary lipids. *Adv. Drug Deliv. Rev.* **50**:21–44 (2001).
2. W. Stremmel. Uptake of fatty acids by jejunal mucosal cells is mediated by a fatty acid binding membrane protein. *J. Clin. Invest.* **82**:2001–2010 (1988).
3. S. L. Chow and D. Hollander. A dual, concentration-dependent absorption mechanism of linoleic acid by rat jejunum *in vitro*. *J. Lipid Res.* **20**:349–356 (1979).
4. W. Stremmel, G. Strohmeyer, F. Borchard, S. Kochwa, and P. D. Berk. Isolation and partial characterization of a fatty acid binding protein in rat liver plasma membranes. *Proc. Natl. Acad. Sci. USA* **82**:4–8 (1985).
5. C. Schoeller, M. Keelan, G. Mulvey, W. Stremmel, and A. B. Thomson. Role of a brush border membrane fatty acid binding protein in oleic acid uptake into rat and rabbit jejunal brush border membrane. *Clin. Invest. Med.* **18**:380–388 (1995).
6. H. Poirier, P. Degrace, I. Niot, A. Bernard, and P. Besnard. Localization and regulation of the putative membrane fatty-acid transporter (FAT) in the small intestine. Comparison with fatty acid-binding proteins (FABP). *Eur. J. Biochem.* **238**:368–373 (1996).
7. S. Lundgren, T. Carling, G. Hjalm, C. Juhlin, J. Rastad, U. Pihlgren, L. Rask, G. Akerstrom, and P. Hellman. Tissue distribution of human gp330/megalin, a putative Ca(2+)-sensing protein. *J. Histochem. Cytochem.* **45**:383–392 (1997).

8. L. B. Agellon, M. J. Toth, and A. B. Thomson. Intracellular lipid binding proteins of the small intestine. *Mol. Cell. Biochem.* **239**:79–82 (2002).
9. N. M. Bass. The cellular fatty acid binding proteins: aspects of structure, regulation, and function. *Int. Rev. Cytol.* **111**:143–184 (1988).
10. P. Besnard, I. Niot, H. Poirier, L. Clement, and A. Bernard. New insights into the fatty acid-binding protein (FABP) family in the small intestine. *Mol. Cell. Biochem.* **239**:139–147 (2002).
11. C. J. Porter and W. N. Charman. Intestinal lymphatic drug transport: an update. *Adv. Drug Deliv. Rev.* **50**:61–80 (2001).
12. G. V. Richieri, R. T. Ogata, A. W. Zimmerman, J. H. Veerkamp, and A. M. Kleinfeld. Fatty acid binding proteins from different tissues show distinct patterns of fatty acid interactions. *Biochemistry* **39**:7197–7204 (2000).
13. A. E. Thumser and J. Storch. Liver and intestinal fatty acid-binding proteins obtain fatty acids from phospholipid membranes by different mechanisms. *J. Lipid Res.* **41**:647–656 (2000).
14. C. Wolfrum, C. Buhlmann, B. Rolf, T. Borchers, and F. Spener. Variation of liver-type fatty acid binding protein content in the human hepatoma cell line HepG2 by peroxisome proliferators and antisense RNA affects the rate of fatty acid uptake. *Biochim. Biophys. Acta* **1437**:194–201 (1999).
15. H. Poirier, I. Niot, P. Degrace, M. C. Monnot, A. Bernard, and P. Besnard. Fatty acid regulation of fatty acid-binding protein expression in the small intestine. *Am. J. Physiol.* **273**:G289–G295 (1997).
16. R. K. Ockner and J. A. Manning. Fatty acid-binding protein in small intestine. Identification, isolation, and evidence for its role in cellular fatty acid transport. *J. Clin. Invest.* **54**:326–338 (1974).
17. R. K. Ockner, J. A. Manning, R. B. Poppenhausen, and W. K. Ho. A binding protein for fatty acids in cytosol of intestinal mucosa, liver, myocardium, and other tissues. *Science* **177**:56–58 (1972).
18. N. Dube, E. Delvin, W. Yotov, C. Garofalo, M. Bendayan, J. H. Veerkamp, and E. Levy. Modulation of intestinal and liver fatty acid-binding proteins in Caco-2 cells by lipids, hormones and cytokines. *J. Cell Biochem.* **81**:613–620 (2001).
19. P. Tso, J. A. Balint, and W. J. Simmonds. Role of biliary lecithin in lymphatic transport of fat. *Gastroenterology* **73**:1362–1367 (1977).
20. C. M. Mansbach 2nd, A. Arnold, and M. A. Cox. Factors influencing triacylglycerol delivery into mesenteric lymph. *Am. J. Physiol.* **249**:G642–G648 (1985).
21. C. M. Mansbach 2nd and R. F. Dowell. Portal transport of long acyl chain lipids: effect of phosphatidylcholine and low infusion rates. *Am. J. Physiol.* **264**:G1082–G1089 (1993).
22. T. T. Kararli. Comparison of the gastrointestinal anatomy, physiology, and biochemistry of humans and commonly used laboratory animals. *Biopharm. Drug Dispos.* **16**:351–380 (1995).
23. N. L. Trevaskis, C. J. Porter, and W. N. Charman. The lymph lipid precursor pool is a key determinant of intestinal lymphatic drug transport. *J. Pharmacol. Exp. Ther.* **316**:881–891 (2006).
24. N. L. Trevaskis, C. J. Porter, and W. N. Charman. Bile increases intestinal lymphatic drug transport in the fasted rat. *Pharm. Res.* **22**:1863–1870 (2005).
25. S. A. Bustin. Quantification of mRNA using real-time reverse transcription PCR (RT-PCR): trends and problems. *J. Mol. Endocrinol.* **29**:23–39 (2002).
26. M. W. Pfaffl. A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res.* **29**:e45, 2001 (2001).
27. B. P. Atshaves, W. B. Foxworth, A. Frolov, J. B. Roths, A. B. Kier, B. K. Oetama, J. A. Piedrahita, and F. Schroeder. Cellular differentiation and I-FABP protein expression modulate fatty acid uptake and diffusion. *Am. J. Physiol.* **274**:C633–C644 (1998).
28. B. P. Atshaves, A. M. McIntosh, O. I. Lyuksyutova, W. Zipfel, W. W. Webb, and F. Schroeder. Liver fatty acid-binding protein gene ablation inhibits branched-chain fatty acid metabolism in cultured primary hepatocytes. *J. Biol. Chem.* **279**:30954–30965 (2004).
29. M. A. Markwell, S. M. Haas, L. L. Bieber, and N. E. Tolbert. A modification of the Lowry procedure to simplify protein determination in membrane and lipoprotein samples. *Anal. Biochem.* **87**:206–210 (1978).
30. H. Poirier, I. Niot, M. C. Monnot, O. Braissant, C. Meunier-Durmort, P. Costet, T. Pineau, W. Wahli, T. M. Willson, and P. Besnard. Differential involvement of peroxisome-proliferator-activated receptors alpha and delta in fibrate and fatty-acid-mediated inductions of the gene encoding liver fatty-acid-binding protein in the liver and the small intestine. *Biochem. J.* **355**:481–488 (2001).
31. J. H. Veerkamp, T. H. van Kuppevelt, R. G. Maatman, and C. F. Prinsen. Structural and functional aspects of cytosolic fatty acid-binding proteins. *Prostaglandins Leukot. Essent Fat. Acids* **49**:887–906 (1993).
32. J. H. Veerkamp and H. T. van Moerkerk. Fatty acid-binding protein and its relation to fatty acid oxidation. *Mol. Cell. Biochem.* **123**:101–106 (1993).
33. M. C. Carey, D. M. Small, and C. M. Bliss. Lipid digestion and absorption. *Annu. Rev. Physiol.* **45**:651–677 (1983).
34. D. R. Prows and F. Schroeder. Metallothionein-IIA promoter induction alters rat intestinal fatty acid binding protein expression, fatty acid uptake, and lipid metabolism in transfected L-cells. *Arch. Biochem. Biophys.* **340**:135–143 (1997).
35. E. L. Holehouse, M. L. Liu, and G. W. Aponte. Oleic acid distribution in small intestinal epithelial cells expressing intestinal-fatty acid binding protein. *Biochim. Biophys. Acta* **1390**: 52–64 (1998).
36. S. Incerpi, J. R. Jefferson, W. G. Wood, W. J. Ball, and F. Schroeder. Na pump and plasma membrane structure in L-cell fibroblasts expressing rat liver fatty acid binding protein. *Arch. Biochem. Biophys.* **298**:35–42 (1992).
37. J. R. Jefferson, D. M. Powell, Z. Rymaszewski, J. Kukowska-Latallo, J. B. Lowe, and F. Schroeder. Altered membrane structure in transfected mouse L-cell fibroblasts expressing rat liver fatty acid-binding protein. *J. Biol. Chem.* **265**:11062–11068 (1990).
38. J. R. Jefferson, J. P. Slotte, G. Nemecz, A. Pastuszyn, T. J. Scallen, and F. Schroeder. Intracellular sterol distribution in transfected mouse L-cell fibroblasts expressing rat liver fatty acid-binding protein. *J. Biol. Chem.* **266**:5486–5496 (1991).
39. F. Schroeder, J. R. Jefferson, D. Powell, S. Incerpi, J. K. Woodford, S. M. Colles, S. Myers-Payne, T. Emge, T. Hubbell, and D. Moncecchi et al. Expression of rat L-FABP in mouse fibroblasts: role in fat absorption. *Mol. Cell. Biochem.* **123**:73–83 (1993).
40. P. Besnard, A. Mallordy, and H. Carlier. Transcriptional induction of the fatty acid binding protein gene in mouse liver by bezafibrate. *FEBS Lett.* **327**:219–223 (1993).
41. G. Hallden, E. L. Holehouse, X. Dong, and G. W. Aponte. Expression of intestinal fatty acid binding protein in intestinal epithelial cell lines, hBRIE 380 cells. *Am. J. Physiol.* **267**: G730–G743 (1994).
42. K. Schoonjans, G. Martin, B. Staels, and J. Auwerx. Peroxisome proliferator-activated receptors, orphans with ligands and functions. *Curr. Opin. Lipidol.* **8**:159–166 (1997).
43. D. A. Bernlohr, M. A. Simpson, A. V. Hertzel, and L. J. Banaszak. Intracellular lipid-binding proteins and their genes. *Annu. Rev. Nutr.* **17**:277–303 (1997).
44. D. H. Alpers, N. M. Bass, M. J. Engle, and K. DeSchryver-Kecskemeti. Intestinal fatty acid binding protein may favor differential apical fatty acid binding in the intestine. *Biochim. Biophys. Acta* **1483**:352–362 (2000).
45. T. Velkov, S. Chuang, J. Wielens, H. Sakellaris, W. N. Charman, C. J. Porter, and M. J. Scanlon. The interaction of lipophilic drugs with intestinal fatty acid-binding protein. *J. Biol. Chem.* **280**:17769–17776 (2005).