

## Investigation of the Assembly of Chylomicrons in Hamster Enterocytes Using Pluronic-L81 Acid as a Probe

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**Abstract-** A major function of the enterocytes is absorption, processing, and export of dietary lipids into the lymphatic system. Pluronic L-81 is a non-ionic hydrophobic surfactant, which specifically inhibits lipid absorption in the intestine when administered *in vivo*. This compound is therefore an attractive probe to perturb and investigate the molecular and intracellular events in chylomicron assembly in the enterocytes. In the present study pluronic acid was administered to hamsters followed by isolation of the enterocytes and cell fractionation to investigate the effect of pluronic acid on intracellular events in lipid absorption. Four types of diet were administered to hamsters for three weeks; low-fat chow, high-fat chow and each diet with or without added pluronic acid. Sub-cellular fractions of freshly isolated enterocytes were prepared. Consistent with morphological observation, the high fat diet resulted in a three-fold increase in the triacylglycerol (TAG) content of the enterocytes and addition of pluronic acid to either the low fat or the high fat diets resulted in a ten-fold increase in cellular TAG levels. Determination of the mass of TAG and the time course of incorporation of <sup>3</sup>H-triolein, administered by gavage, showed that the increased TAG was recovered in the microsomal (endoplasmic reticulum) fraction and the cytosol. In microsomes, increased TAG was recovered mainly in the membrane although there was a significant amount in the microsomal luminal contents. Pluronic acid therefore acts at the level of the endoplasmic reticulum and inhibits the assembly of apo-B48 with chylomicron TAG. The excess TAG is transferred to cytosolic stores.

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### Introduction

One of the major functions of the small intestinal mucosal cell, enterocyte, is absorption, processing, and export of dietary lipids (1). Dietary lipids, which cannot be absorbed by the enterocytes, are first emulsified in the jejunal lumen to very small lipid droplets and then transferred across the brush border of the enterocytes (2,3). The triglyceride is reformed in the endoplasmic reticulum and packaged with phospholipid, cholesterol, cholesterol esters and protein to form chylomicrons, which are secreted at the lateral borders of the enterocytes. The major protein essential for the assembly of chylomicrons is apo-B48. This is characteristic of the enterocytes and is formed by post-translational editing of the apo-B100 mRNA, so that a truncated form of apo-B is formed (1,2). The full length protein, apo-B100, is characteristic of very low density lipoproteins, which are the vehicle of transport of

endogenous lipids and secreted by the liver. Enterocytes are highly polarized, tall, columnar cells with a general architecture and structure, which is similar to a number of other epithelial cell types. These cells process large amounts of triacylglycerol (TAG) during fat-containing meals, and package the TAGs together with proteins, phospholipids and cholesterol into lipoprotein droplets, chylomicrons. By the process of the exocytosis, chylomicrons are secreted into the chyme of the lymphatic system by the enterocytes, and enter the blood via the thoracic duct (4).

Because of the unavailability of normal human intestinal epithelial cell lines, most of our knowledge about human intestinal function has been derived from Caco2 (human colon carcinoma) cell line. This cell line was used as a model to study intestinal lipid metabolism for the first time by Hughes *et al.*, (3,4) and Traber *et al.*, (5) and has been used extensively to investigate the regulation of intestinal lipoprotein secretion and other

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functions (6-12). However, theoretical limitations with these models are obviously their cancerous nature and their colonic origin. Moreover these cell lines secrete particles of the density of VLDL/LDL containing both apoB-100, which is characteristic of liver and fetal intestine, and apoB-48, which is characteristic of adult intestine (5-7). Furthermore it is not possible to investigate the effects of diet manipulation on the assembly of chylomicrons by cultured cells.

Recently Pereault, and Beaulieu have used specimens of small intestine (jejunum and ileum) from fetuses at 12 to 20 weeks of gestation, to isolate viable enterocytes (8,9). These differ from adult intestine in function; moreover these specimens are from jejunum and ileum although most part of the lipid absorption occurs in the one quarter of upper small intestine, duodenum. Due to the above disadvantages, for the first time Cartwright and Higgins isolated rabbit enterocytes (10), and then we have used of this method to isolate freshly hamster enterocytes.

## Materials and Methods

In order to investigate synthesis, intracellular fate and secretion of newly synthesized chylomicrons, and to consider effects of dietary manipulation on the assembly and secretion of chylomicrons in enterocytes in the system as close to the physiological normal as possible we have used freshly isolated hamster enterocytes from different diet hamsters prepared by the method of Cartwright and Higgins (10). After homogenization of enterocytes, sub-cellular fractions, microsomes and cytosol, were prepared by several centrifugations from cell homogenate by method of Weiser (11,12).

### Materials

Pluronic acid L81 was a gift from Dr Patrick Tso, University of Cincinnati, USA). Hamsters (DSNI strain) were provided by Dr A. M. Salter, University of Nottingham, Sutton Bonnington, UK). All other reagents and materials were as described previously (17-19,28,29).

### Animals

Male Golden Syrian hamsters, (DSNI), between 120-180g were used in this study. They were housed in the Sheffield University animal field laboratories and were maintained on Rodent Maintenance diet 3 (RM3) (Special Diet Services, Dietex International, Witham, Essex CM8 3AD, UK). They were allowed free access

to food and water, and the light cycle was 0600-1800hr. Animals were sacrificed between 9.30-10 am.

### Diets

RM3 was supplemented as followed and administered for three weeks, (a) chow fed control RM3, (b) RM3 + fat of the composition of a typical western diet (11.4% lard, 3% sunflower oil, 3% tripalmitin, w/w/w), (c) RM3 + pluronic acid (1% for the first week and 0.5% for weeks 2 and 3), (d) high-fat diet (b) + pluronic acid (0.1% for first week, 0.5% for weeks 2 and 3). In some experiments, after dietary manipulation, hamsters were gavaged with 10 $\mu$ Ci <sup>3</sup>H triolein dissolved in 0.1ml sunflower oil and killed after 30min, 1 hour, 2 hours or 3 hour.

### Isolation of enterocytes and subcellular fractionation

Enterocytes were isolated from all control and experimental hamsters using the method developed for rabbit enterocytes (17). Sub-cellular fractions (microsomes and cytosol) were prepared from isolated enterocytes as previously described (18, 19). Yields of isolated enterocytes were insufficient to do further cell fractionation, however, the microsomes consist predominantly of endoplasmic reticulum, which is the site of chylomicron assembly in rabbit (18,19). Membrane and luminal sub-fractions were prepared from total microsomes using sodium carbonate as previously described (18,19).

### Determination of apo-B48

Aliquots of all samples (50 $\mu$ g of cells, microsomes and microsomal membranes and 75 $\mu$ g of luminal contents) were separated by gel electrophoresis on 3-20% gels and transferred to nitrocellulose membranes as described previously (17-19). Apo-B48 was detected by western blotting and quantified using a scanner equipped with BioRad quantity 1 Software (version 3.2) as described by Bergeron, Kotite and Havel (1996) (30).

### Lipid analysis

Lipids were extracted from all cell and sub-cellular fractions and separated by high performance thin layer chromatography (HPTLC) or by conventional thin layer chromatography (TLC) as described previously (17-19,28). In experiments in which <sup>3</sup>H-triolein was gavaged, lipids were separated by TLC and the lipid containing bands were scrapped from the TLC plate and the radioactivity determined using a TRI-CARB Packard scintillation counter.

**Analysis of plasma lipoproteins**

Plasma lipoprotein TAG and cholesterol profiles were determined using hamster plasma and iodixanol gradient technology as described previously by Sawle *et al.* (29).

**Data analysis**

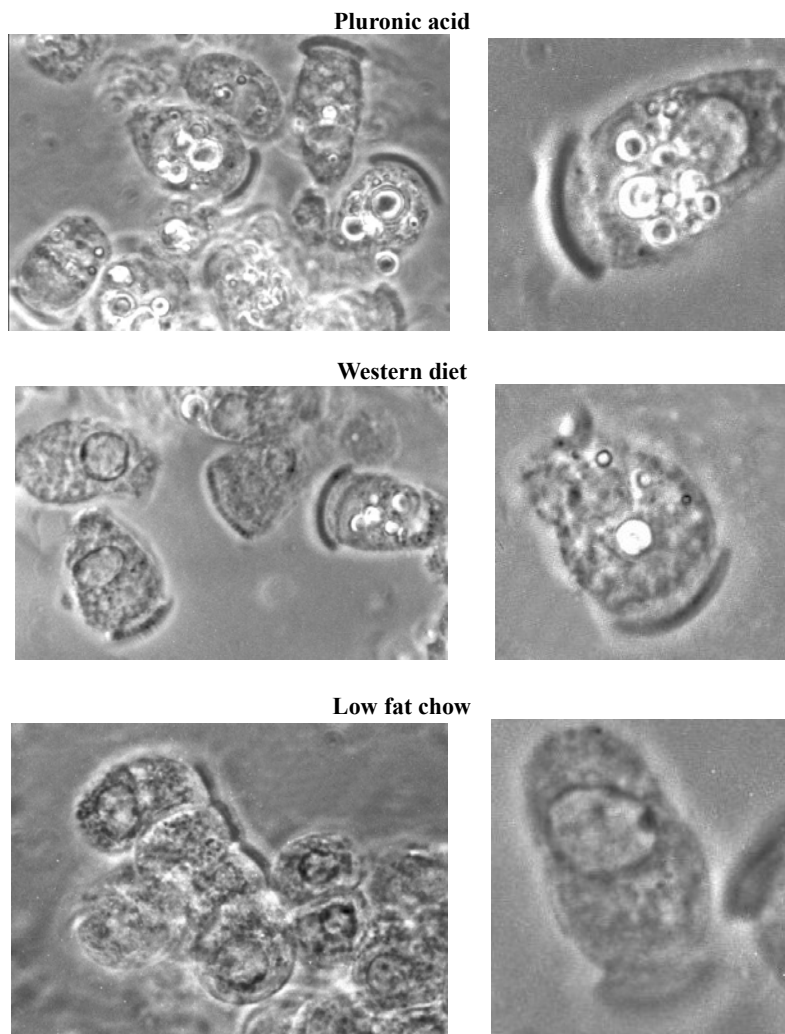
Statistical analysis was carried out using the paired student's t-test for comparison of means in parametrical data and Mann-Whitney test for independent non-parametrical dates. One-Way ANOVA (Analysis of Variance) were used to compare the effect of different diets on lipid pools. Computations were done using Microsoft Excel 98 software, SPSS 12.01, and STATA

programmes. (Data was deemed significant when  $P < 0.05$ ).

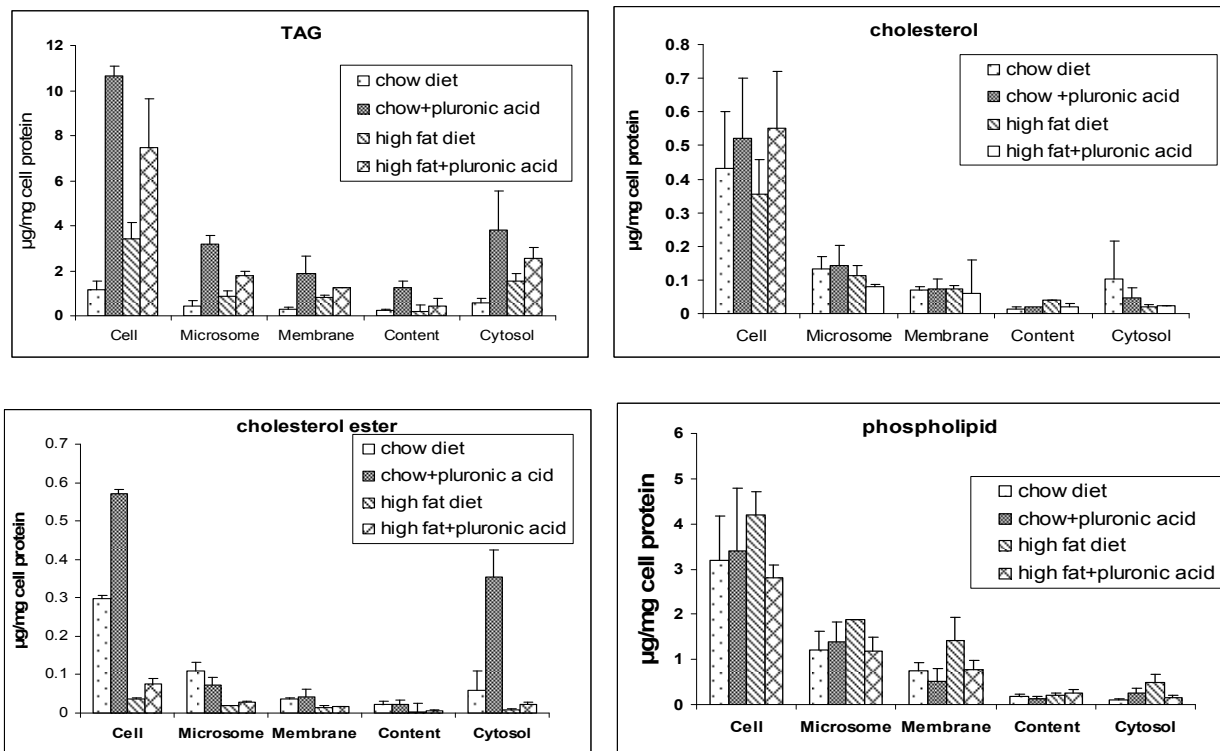
**Results**

**Morphology of isolated enterocytes from animals fed different diets**

Isolated enterocytes exhibited the characteristic morphology of enterocytes *in situ*. These were columnar cells with a marked brush border, with a tendency to adhere in sheets consisting of a number of cells. The preparations consisted of only enterocytes with a high degree of purity (Figure 1).



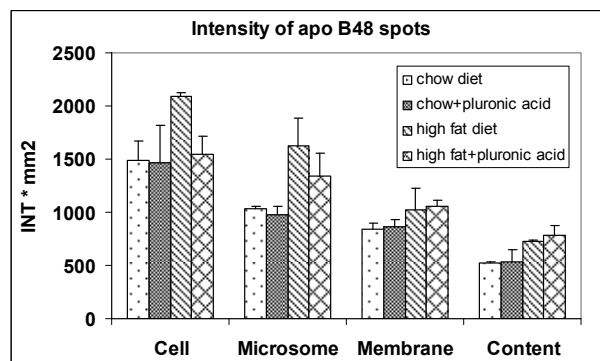
**Figure 1.** The morphology of enterocytes isolated from hamsters fed low fat chow, low fat chow plus pluronic acid, high fat diet and high fat plus pluronic acid



**Figure 2.** Effect of pluronic acid feeding on the lipids of enterocytes, microsomes, microsomal subfractions and cytosol. Hamsters were fed the four diets for three weeks as described in Methods. Lipids were extracted from the cells and sub-cellular fractions, separated by HPTLC and the TAG, cholesterol, cholesterol ester and phospholipids determined as described in Methods. Results are expressed in terms of protein of the original cells and are the average of six determinations  $\pm$  SEM.

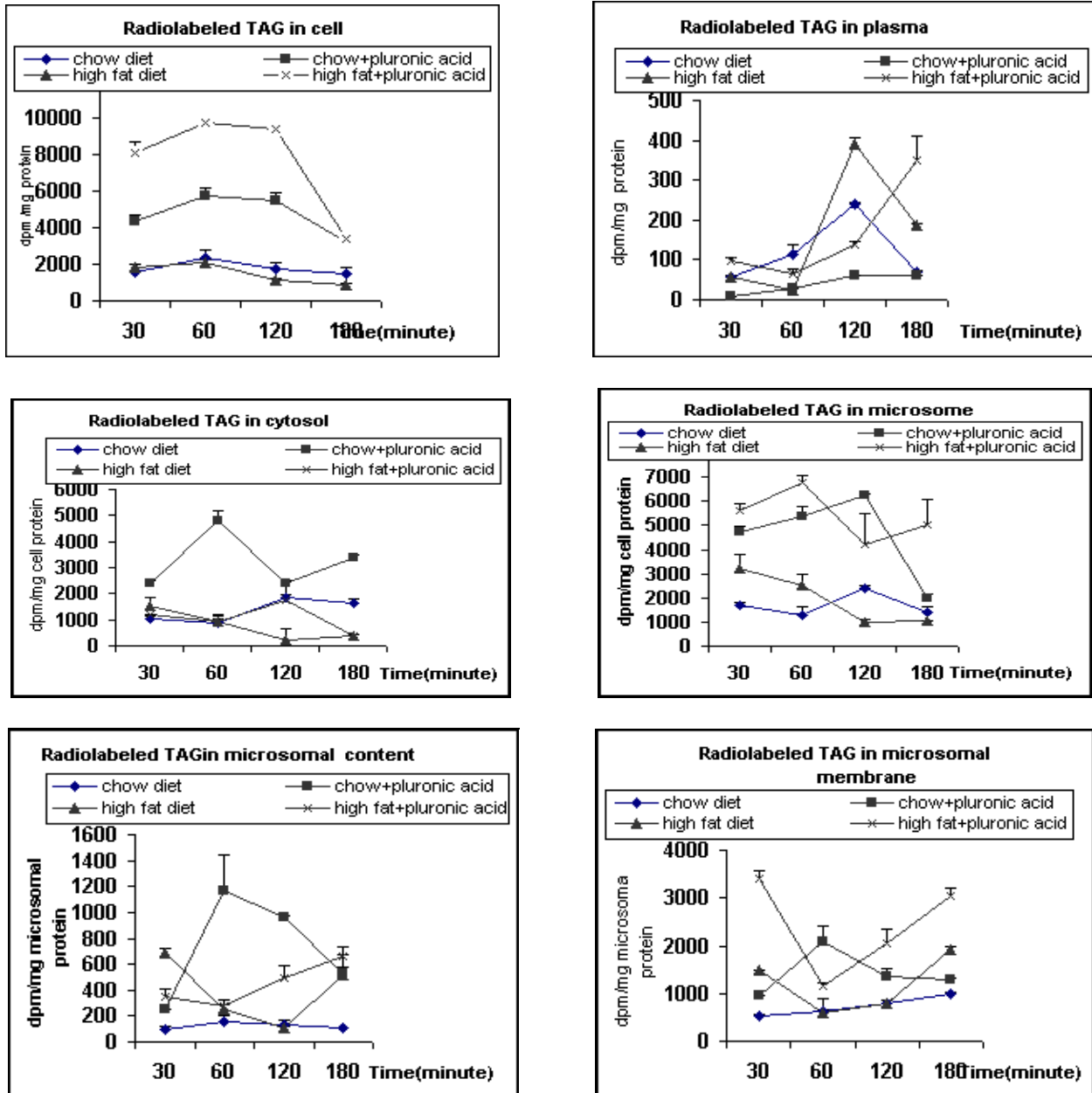
Enterocytes prepared from hamsters fed a high-fat diet showed a moderate accumulation of lipid droplets in the cytoplasm. However, feeding a chow diet or a high fat diet containing pluronic acid resulted in accumulation of large amounts of lipid droplets in the cytoplasm (Figure 1). Pluronic acid feeding resulted in accumulation of TAG in the enterocytes. Feeding a high fat diet increased the TAG content of enterocytes approximately three fold compared with the control chow diet ( $P < 0.05$ ). Addition of pluronic acid to the chow diet resulted in a ten-fold increase in the TAG of the enterocytes ( $P < 0.05$ ). The TAG content of the enterocytes fed a high fat diet + pluronic acid was similar to that of low fat diet + pluronic acid. This may be because the cells can only accumulate a maximum amount of TAG so that the high fat diet and pluronic acid diet do not have accumulative effects. The addition of pluronic acid to low fat chow or a high fat diet resulted in accumulation of TAG in the cytosol fraction ( $P < 0.05$  for all), and in the microsomes ( $P < 0.05$  for all). When the microsomes were separated into membranes and luminal contents, accumulation of TAG was greater in the membrane fraction, but showed similar patterns to the microsomes, with the greatest effect in the case of

the pluronic acid fed animals.



**Figure 3.** Amount of apo-B48 in enterocytes and subcellular fractions.

Hamsters were fed the four diets for three weeks as described in Methods. Proteins (50µg of cell, microsome and membrane protein and 75µg of luminal content protein) were separated by SDS-PAGE electrophoresis on 3-20% gels and transferred by electroblotting to nitrocellulose sheets. The apo-B48 was detected by western blotting and the amount of apo-B48 in all fractions quantified and expressed in arbitrary units of intensity, as described in Methods. Results are the average of four determinations  $\pm$  SEM.



**Figure 4.** Effect of pluronic acid on the incorporation of <sup>3</sup>H triolein into lipids of enterocytes, plasma and subcellular fractions. Hamsters were fed the four diets and <sup>3</sup>H-triolein was administered by gavage as described in Methods. Enterocytes were prepared from all animals at a range of times after gavage. Lipids were extracted from the cells and subcellular fractions as described in Methods. Blood was taken immediately prior to removal of the intestine, red cells were pelleted and lipids were extracted from 1ml of the plasma. Lipids from all samples were separated by TLC, and the lipid containing bands scrapped from the plates and the radioactivity determined. Results are expressed in terms of the cell protein in the case of enterocytes, plasma and cytosol and in terms of microsomal protein in the case of microsomes, membrane and content subfractions.

The cholesterol ester content of the enterocytes from hamsters fed chow was approximately six fold greater than that of enterocytes fed a high fat diet ( $P < 0.05$ ). This simplest explanation for this result is that a high fat diet (which is not enriched with cholesterol) stimulates chylomicron secretion by the enterocytes and therefore

clears cholesterol ester from the cells. Addition of pluronic acid to either chow diet or a high fat diet results in an increase of about two fold in the cholesterol ester content of the enterocytes ( $P < 0.05$ ). Most of the cholesterol ester that accumulates after addition of pluronic acid to the low fat chow is recovered in the

cytosolic fraction ( $P < 0.05$ ). There was no significant change in the cholesterol or phospholipids content of enterocytes or subcellular fractions prepared from hamsters fed any of the four diets.

Pluronic acid feeding has little effect on the apo-B48 content of enterocytes. There was a small non-statistically significant increase in the apo-B48 content of enterocytes fed a high fat diet compared with chow fat chow and similar increase in the apo-B48 of microsomes (Figure 3). Addition of pluronic acid to a chow diet or a high fat diet had no significant effect on the quantity of apo B48 detected by western blotting and quantified (Figure 3). Thus the accumulation of TAG in the cells from animals fed pluronic acid is not accompanied by increases in apo-B48 which is essential for the assembly and secretion of chylomicrons.

#### **Incorporation of 3H triolein administered in vivo**

To determine the kinetics of incorporation of 3H-triolein into enterocytes this lipid was administered by gavage and the enterocytes and plasma were isolated at a range of times. Triolein is hydrolysed in the lumen of the small intestine, absorbed across the brush border and the TAG is reesterified. This then moves between intracellular compartments and is secreted in chylomicrons, which move from the lymph to the plasma. The time course of intracellular transit and the relationships between the different lipid pools are therefore extremely complex.

#### **Pluronic acid treatment increases the radio-labelled TAG content of enterocytes**

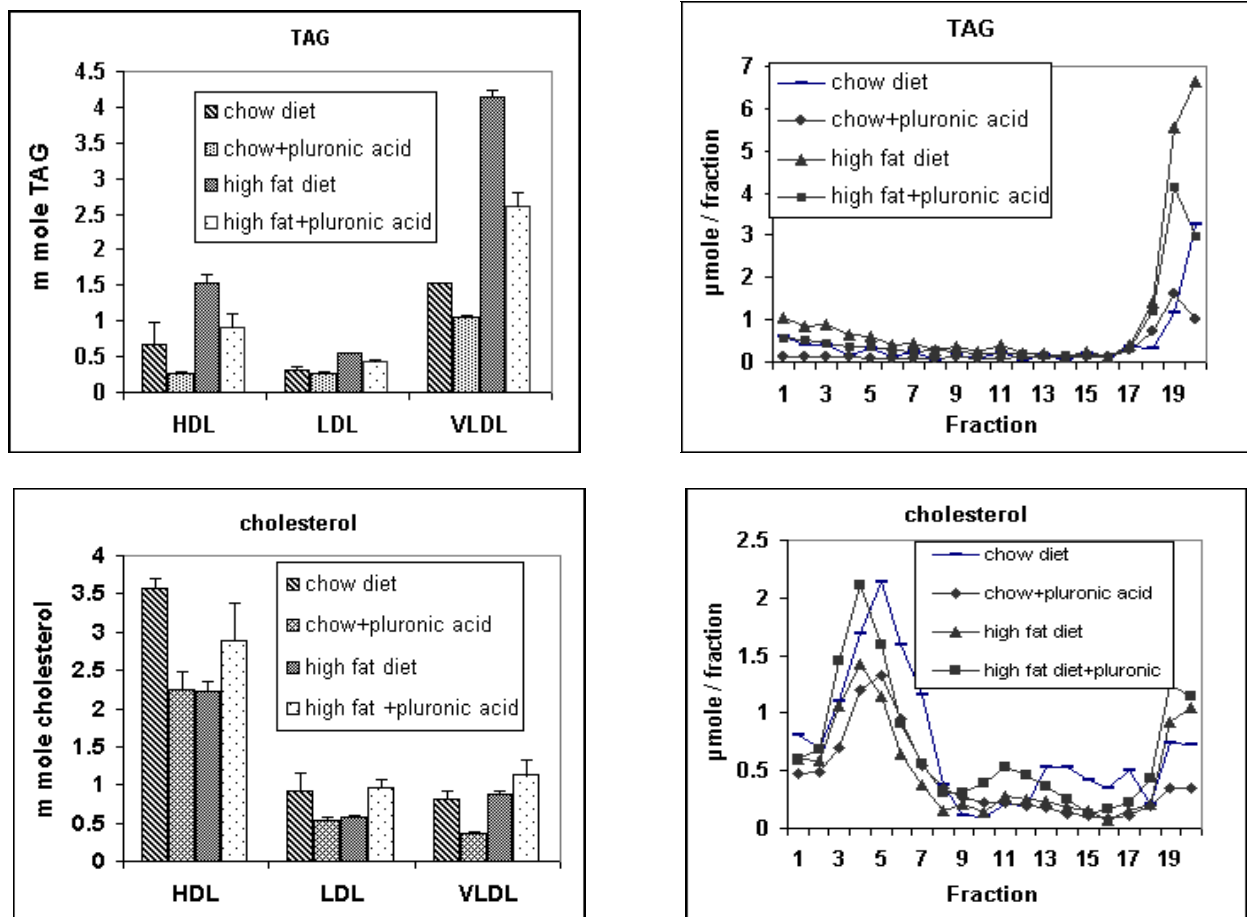
Over a time period of 180 min from the administration of 3H-triolein the appearance of radiolabelled TAG in enterocytes from hamsters fed chow diets or high fat diets was similar (Figure 4a). Addition of pluronic acid to either diet resulted in an increase in radiolabelled TAG in the enterocytes for the first 120 min after diet administration after which the radioactivity fell (Figure 4a). Radiolabelled TAG was greater in the hamsters fed a high fat diet + pluronic acid compared with a chow diet + pluronic acid. These results confirm those from measurements of the mass of TAG and demonstrate that pluronic acid causes accumulation of dietary administered TAG. They also demonstrate that inhibition by pluronic acid under the experimental conditions is not complete, especially with a high fat diet, but significantly slowed.

Pluronic acid decreases appearance of radiolabelled TAG in the plasma. In parallel the appearance of 3H-triolein in plasma was also measured. In this case there

was a lag period of about 60 min, when appearance of radiolabelled TAG was low (Figure 4b). After 120min hamsters fed low fat chow or the high fat diet showed the greatest accumulation of radiolabelled TAG compared with the same diet with addition of pluronic acid (Figure 4b). After 180 min the radiolabelled TAG in the chow fed or high fat fed hamster plasma fell, presumably because the chylomicrons were cleared (Figure 4b). At the same time the radiolabelled TAG in the high fat diet increased, confirming that inhibition of chylomicron accumulation is not complete especially in the case of a high fat diet. The radiolabelled TAG in the plasma of chow + pluronic acid fed hamsters remained low. Pluronic acid increases the appearance of radiolabelled TAG in microsomes, membrane and content fractions. At all time points addition of pluronic acid to either a chow diet or high fat diet increases the appearance of radiolabelled triolein in microsomes (Figure 4c). In chylomicron assembly the TAG is initially incorporated into the microsomal membrane and then transferred to the lumen and assembled into chylomicrons. As the apo-B48 content of the enterocytes is not significantly increased with the four diets one aim of this study was to determine whether TAG remains in the membrane or whether increased apo-B48 is necessary for its transfer to the lumen. Addition of pluronic acid to the low fat chow increases radiolabelled TAG in the membranes and the luminal content (Figure 5e and f). This indicates that pluronic acid slows but does not prevent the transfer of TAG from the membrane to the lumen.

With the high fat diet the patterns of incorporation of 3H triolein were different. After an initial high incorporation the radiolabelled TAG fell and then rose more slowly (Figure 5 e and f). On addition of pluronic acid to the high fat diet radiolabelled TAG was increases in both the membrane and the luminal content. Overall these results suggest that pluronic acid inhibits both the movement of TAG from the membrane to the lumen and the assembly of chylomicrons.

Pluronic acid increases incorporation of radiolabelled TAG in the cytoplasm. TAG in excess to that required for chylomicron assembly is transferred to cytosolic stores. Newly synthesised TAG accumulates in the cytosol to the greatest extent in the case of hamsters fed chow diet + pluronic acid. This is probably a consequence of the intracellular dynamics of the TAG pools. A high fat diet overcomes the inhibitory effect of pluronic acid to some extent and allows some chylomicron secretion. However, pluronic acid added to a low fat chow results in greater inhibition of



**Figure 5.** Analysis of plasma of hamsters fed the four diets.

Hamsters were fed the four different diets. Plasma was removed by cardiac puncture, the red cells were pelleted by centrifugation and the TAG and cholesterol profiles were determined using iodixanol gradients as described in Methods.  $\mu$ Moles per fraction (TAG or cholesterol) are plotted against the fraction. A typical gradient is plotted for each diet on the left. In the graphs on the right the HDL, LDL and VLDL were calculated from the gradients and the average of four determinations are plotted  $\pm$  SEM.

chylomicron secretion and the excess TAG is transferred to the cytosol.

**Dietary pluronic acid lowers the TAG of plasma lipoproteins**

To investigate the effect of the different diets on plasma lipoproteins, TAG and cholesterol profiles (HDL, LDL and VLDL) were determined using iodixanol gradients. Plasma TAG, particularly that of VLDL, was increased by feeding a high fat diet. Addition of pluronic acid to low fat chow or high fat diets reduced the TAG in all lipoprotein fractions. These observations are consistent with the inhibitory effect of pluronic acid on absorption of dietary TAG. The cholesterol content of HDL, LDL and VLDL was reduced by addition of pluronic acid to a low fat diet. However, addition of pluronic acid to a high fat diet increased the cholesterol of all lipoproteins.

**Discussion**

In this study the enterocytes of hamsters were prepared using the method developed for rabbit enterocytes by Cartwright and Higgins (10). The morphology of the enterocytes differs depending on the diets. A fat enriched diet results in accumulation of fat droplets in the cells; this is very striking after pluronic acid feeding and suggests that this treatment results in a block in chylomicron assembly as reported by other investigators (16-18).

According to these results most protein content of cell homogenate appears in the cytosol. This might be due to the fact that, in most tissues a substantial proportion of the protein of the cell is in the cytosol (19). 52%, 54%, 56.8%, and 56.9% of protein was in the luminal content of microsomal pellet of different diets (RM3, RM3+pluronic acid, RM3+western oil, and

RM3+western oil+pluronic acid respectively). These results are compatible with previously published work from this laboratory and other laboratory using rat or rabbit liver (20-22), they found 40-54.5% of the protein was recovered in the cisternal contents.

Dietary manipulations had little effect on the protein content of cell homogenate or sub-cellular fractions ( $P>0.05$ ), which is compatible with Wilkinson work, and Iddon results for the liver (23,24).

To determine the purity of the microsomal fraction compared with whole homogenate, NADPH cytochrome C reductase was used. Results of these assay were shown in table 1, the microsomal prepared in this study contained 31.5%, 36%, 22.33%, and 24.33% of the endoplasmic reticulum, determined by recovery of the NADPH cytochrome C reductase for RM3, RM3+pluronic acid, RM3+western oil, and RM3+western oil+pluronic acid diets respectively. Enrichment was 3.33, 3.2, 2.23, and 1.93 fold higher specific activity of NADPH cytochrome C reductase than the whole homogenate for RM3, RM3+pluronic acid, RM3+western oil, and RM3+western oil+pluronic acid diets hamsters respectively.

Plonne *et al.*, have found 53% recovery of NADPH cytochrome C reductase in microsomal pellet from isolated hepatocytes (25), also this enzyme activity recovery were 30%, and 31% for the microsomal pellets prepared from rabbit liver and rabbit enterocytes respectively by Cartwright, *et al.* (26), Cartwright, Plonne, and Higgins (12), In study of Polheim, *et al.* (36) on the intestinal microsomes 28% recovery was observed.

In intestinal enterocytes Mansbach *et al.*, found a 3.6 fold increase in the specific activity of NADPH

cytochrome C reductase as compared to the whole homogenate (37).

In the present we found enrichment around 3 fold in the microsomes compared with the homogenizing cell.

Furthermore increases in the enrichment of RM3 and RM3+pluronic acid diet in compare with RM3+western oil and RM3+western oil+pluronic acid diets are not significant ( $P>0.05$ ), which means diet manipulation have no significant effect on the enrichment of microsomal pellet. Moreover results have shown adding pluronic acid to RM3 (normal) diet can increase recovery of NADPH cytochrome C reductase in the microsomes ( $P=0.02$ ) although this result is not found for RM3+western oil, high fat diet, ( $P=0.2$ ). On the other hand, after adding western oil, high fat, into RM3 diet with or without pluronic acid recovery of the microsomal pellets was significantly decreased ( $P<0.006$ , and  $P<0.003$ , for RM3 compare by RM3+western oil, and RM3 compare by RM3+western oil+pluronic acid respectively).

The observations reported here indicate that pluronic acid inhibits chylomicron assembly and secretion but does not affect the initial stages of dietary TAG hydrolysis in the intestinal lumen, transfer into the enterocytes and re-esterification. This is consistent with the reports from Tso and co workers (22-27) and more recent work from other laboratories (12,31) and makes pluronic acid a useful tool to investigate the molecular and intracellular events in chylomicrons assembly. The effect of addition of pluronic acid to a low fat chow diet or a high fat diet is accumulation of TAG in the isolated enterocytes. In contrast cholesterol ester of enterocytes is increased by addition of pluronic acid to a low fat diet but is reduced by a high fat diet with or without pluronic acid.

**Table 1.** The distribution of NADPH-cytochrome C reductases in microsome fraction from isolated enterocytes of different hamsters

Fraction	Specific activity	Enrichment	Yield
Homogenate(R)	0.092±0.006		
Microsome(R)	0.2085±0.007	3.33± 0.95	31.5±2.4
Homogenate(R+P)	0.0804±0.001		
Microsome(R+P)	0.210±0.002	3.2±0.46	36 ±0.0
Homogenate(R+W)	0.083±0.04		
Microsome(R+W)	0.132±0.07	2.23± 0.42	22.33±2.9
Homogenate(R+W+P)	0.114±0.001		
Microsome(R+W+P)	0.202±0.0004	1.93± 0.78	24.33±0.

The values are given as means± standard deviations. Results are expressed as nmole of reduced cytochrome c / min / mg protein (specific activity). Enrichment is the specific activity of the fraction divided by that for homogenate, and the yield is the % of the total activity recovered in each fraction as a % of that in the homogenate.

R= Rm3 diet (4), R+P= RM3+Pluronic acid diet (3), R+W = RM3+western oil diet (3), R+W+P = RM3+western oil+pluronic acid (3).

The number of determination is given in brackets.



The different diets are not enriched with cholesterol and we conclude that a high fat diet overcomes the effect of pluronic acid to some extent and stimulates secretion of cholesterol ester in chylomicrons. This is supported by the observation that radio-labelled TAG appears in the plasma of hamsters fed a high fat diet + pluronic acid but not a low fat diet + pluronic acid.

Our previous observations indicate that chylomicron assembly takes place in two steps. Apo-B48 is synthesised by bound ribosomes. However, this protein remains membrane bound and moves to the smooth endoplasmic reticulum where it is transferred from the membrane to the lumen with a small amount of phospholipid to form small dense particles (18, 19). TAG is synthesised in the smooth endoplasmic reticulum by esterification of the monoglyceride which is absorbed from the lumen of the small intestine. The small dense apo-B48 containing particles fuse with the membrane bound TAG to form chylomicrons. In the chow fed animal the rate limiting step is the transfer of the TAG to the lumen (19) and in the fat fed animal the rate limiting step is the transfer of the assembled chylomicron from the endoplasmic reticulum to the Golgi for secretion (19). These observations are consistent with the reports of Mansbach and colleagues who reported a rate limiting step from endoplasmic reticulum to Golgi in chylomicron assembly and secretion (19,32-35). In the hepatocyte, during VLDL assembly, TAG rich particles without apo-B48 are formed as an intermediate; however, such particles were not detected in the enterocyte in our previous studies (18,19). The purpose of the present study was to investigate the intracellular sites of TAG in the enterocytes, the effect that pluronic acid has on these TAG pools and the movement of newly synthesised TAG between these pools.

Pluronic acid treatment had no significant effect on the amount of apo-B48 in the enterocytes, despite the fact that the cellular TAG pool was increased tenfold. We have pin-pointed the intracellular site of action of pluronic acid as the endoplasmic reticulum, the site of TAG synthesis. Overall the results suggest that pluronic acid inhibits the fusion of apo-B48 containing dense particles with TAG droplets. The endoplasmic reticulum membrane can only hold a certain amount of TAG and this either moves into the lumen or into the cytosolic TAG droplets. When the microsomes were separated into membrane and luminal content fractions we found that most of the accumulated TAG in response to pluronic acid feeding was in the membrane but there

was some accumulation in the luminal contents. To examine this further we fed radio-labelled triolein to trace newly synthesised TAG. In this case, radio-labelled TAG accumulated after pluronic acid feeding in the microsomes and the greatest amount of radio-label was in the membrane. However, in the hamsters fed low fat chow pluronic acid caused a relatively large accumulation of the radio-labelled TAG in the microsomal lumen. However, there was also accumulation of radio-labelled TAG in the cytosol suggesting that pluronic acid inhibits transfer of TAG from the membrane and the luminal contents and the excess is transferred to cytosolic stores.

In summary, pluronic acid treatment causes accumulation of TAG in the endoplasmic reticulum but does not increase the apo-B48 pool. Most of the increased TAG is in the membrane and the excess TAG is transferred to the cytosol and to a smaller extent to the lumen of the endoplasmic reticulum. A high fat diet partly overcomes the inhibition by pluronic acid and allows some chylomicron assembly and secretion.

## Acknowledgement

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## References

1. Powell LM, Wallis SC, Pease RJ, Edwards YH, Knott TJ, Scott J. A novel form of tissue-specific RNA processing produces apolipoprotein-B48 in intestine. *Cell* 1987; 50(6):831-40.
2. Chen SH, Habib G, Yang CY, Gu ZW, Lee BR, Weng SA, Silberman SR, Cai SJ, Deslypere JP, Rosseneu M. Apolipoprotein B-48 is the product of a messenger RNA with an organ-specific in-frame stop codon. *Science* 1987; 238(4825):363-6.
3. Hughes TE, Sasak WV, Ordovas JM, Forte TM, Lamon-Fava S, Schaefer EJ. A novel cell line (Caco-2) for the study of intestinal lipoprotein synthesis. *J Biol Chem* 1987; 262(8):3762-7.
4. Hughes TE, Ordovas JM, Schaefer EJ. Regulation of intestinal apolipoprotein B synthesis and secretion by Caco-2 cells. Lack of fatty acid effects and control by intracellular calcium ion. *J Biol Chem* 1988; 263(7):3425-31.
5. Traber MG, Kayden HJ, Rindler MJ. Polarized secretion of newly synthesized lipoproteins by the Caco-2 human intestinal cell line. *J Lipid Res* 1987; 28(11):1350-63.

6. Hidalgo IJ, Raub TJ, Borchardt RT. Characterization of the human colon carcinoma cell line (Caco-2) as a model system for intestinal epithelial permeability. *Gastroenterology* 1989; 96(3):736-49.
7. Trotter PJ, Storch J. Fatty acid uptake and metabolism in a human intestinal cell line (Caco-2): comparison of apical and basolateral incubation. *J Lipid Res* 1991; 32(2):293-304.
8. Hussain MM, Kancha RK, Zhou Z, Luchoomun J, Zu H, Bakillah A. Chylomicron assembly and catabolism: role of apolipoproteins and receptors. *Biochim Biophys Acta* 1996; 1300(3):151-70.
9. Luchoomun J, Hussain MM. Assembly and secretion of chylomicrons by differentiated Caco-2 cells. Nascent triglycerides and preformed phospholipids are preferentially used for lipoprotein assembly. *J Biol Chem* 1999; 274(28):19565-72.
10. Hussain MM. A proposed model for the assembly of chylomicrons. *Atherosclerosis* 2000; 148(1):1-15.
11. Hussain MM, Kedees MH, Singh K, Athar H, Jamali NZ. Signposts in the assembly of chylomicrons. *Front Biosci* 2001; 6:D320-31.
12. Engle MJ, Mahmood A, Alpers DH. Regulation of surfactant-like particle secretion by Caco-2 cells. *Biochim Biophys Acta* 2001; 1511(2):369-80.
13. Perreault N, Jean-François B. Use of the dissociating enzyme thermolysin to generate viable human normal intestinal epithelial cell cultures. *Exp Cell Res* 1996; 224(2):354-64.
14. Perreault N, Beaulieu JF. Primary cultures of fully differentiated and pure human intestinal epithelial cells. *Exp Cell Res* 1998; 245(1):34-42.
15. Boucher MJ, Rivard N. Regulation and role of brush border-associated ERK1/2 in intestinal epithelial cells. *Biochem Biophys Res Commun* 2003; 311(1):121-8.
16. Weiser MM. Intestinal epithelial cell surface membrane glycoprotein synthesis. II. Glycosyltransferases and endogenous acceptors of the undifferentiated cell surface membrane. *J Biol Chem* 1973; 248(7):2542-8.
17. Cartwright IJ, Higgins JA. Isolated rabbit enterocytes as a model cell system for investigations of chylomicron assembly and secretion. *J Lipid Res* 1999; 40(7):1357-65.
18. Cartwright IJ, Plonné D, Higgins JA. Intracellular events in the assembly of chylomicrons in rabbit enterocytes. *J Lipid Res* 2000; 41(11):1728-39.
19. Cartwright IJ, Higgins JA. Direct evidence for a two-step assembly of ApoB48-containing lipoproteins in the lumen of the smooth endoplasmic reticulum of rabbit enterocytes. *J Biol Chem* 2001; 276(51):48048-57.
20. Kendrick JS, Wilkinson J, Cartwright IJ, Lawrence S, Higgins JA. Regulation of the assembly and secretion of very low density lipoproteins by the liver. *Biol Chem* 1998; 379(8-9):1033-40.
21. Higgins JA. Assembly of triglyceride-transporting plasma lipoproteins. In: Zemleni J, Daniel H, editors. *Molecular Nutrition*. Wallingford, UK: CABI Publishing; 2003. p. 293-300.
22. Tso P, Balint JA, Rodgers JB. Effect of hydrophobic surfactant (Pluronic L-81) on lymphatic lipid transport in the rat. *Am J Physiol* 1980; 239(5):G348-53.
23. Tso P, Balint JA, Bishop MB, Rodgers JB. Acute inhibition of intestinal lipid transport by Pluronic L-81 in the rat. *Am J Physiol* 1981; 241(6):G487-97.
24. Tso P, Buch KL, Balint JA, Rodgers JB. Maximal lymphatic triglyceride transport rate from the rat small intestine. *Am J Physiol* 1982; 242(4):G408-15.
25. Tso P, Gollamudi SR. Pluronic L-81: a potent inhibitor of the transport of intestinal chylomicrons. *Am J Physiol* 1984; 247(1 Pt 1):G32-6.
26. Tso P, Balint JA. Formation and transport of chylomicrons by enterocytes to the lymphatics. *Am J Physiol* 1986; 250(6 Pt 1):G715-26.
27. Nutting D, Hall J, Barrowman JA, Tso P. Further studies on the mechanism of inhibition of intestinal chylomicron transport by Pluronic L-81. *Biochim Biophys Acta* 1989; 1004(3):357-62.
28. Cartwright IJ, Higgins JA. Increased dietary triacylglycerol markedly enhances the ability of isolated rabbit enterocytes to secrete chylomicrons: an effect related to dietary fatty acid composition. *J Lipid Res* 1999; 40(10):1858-66.
29. Sawle A, Higgins MK, Olivant MP, Higgins JA. A rapid single-step centrifugation method for determination of HDL, LDL, and VLDL cholesterol, and TG, and identification of predominant LDL subclass. *J Lipid Res* 2002; 43(2):335-43.
30. Bergeron N, Kotite L, Havel RJ. Simultaneous quantification of apolipoproteins B-100, B-48, and E separated by SDS-PAGE. *Methods Enzymol* 1996; 263:82-94.
31. Morita SY, Kawabe M, Nakano M, Handa T. Pluronic L81 affects the lipid particle sizes and apolipoprotein B conformation. *Chem Phys Lipids* 2003; 126(1):39-48.
32. Kumar NS, Mansbach CM. Determinants of triacylglycerol transport from the endoplasmic reticulum to the Golgi in intestine. *Am J Physiol* 1997; 273(1 Pt 1):G18-30.
33. Kumar NS, Mansbach CM 2nd. Prechylomicron transport vesicle: isolation and partial characterization. *Am J Physiol* 1999; 276(2 Pt 1):G378-86.
34. Mansbach CM 2nd, Nevin P. Intracellular movement of triacylglycerols in the intestine. *J Lipid Res* 1998; 39(5):963-8.

35. Mansbach CM, Dowell R. Effect of increasing lipid loads on the ability of the endoplasmic reticulum to transport lipid to the Golgi. *J Lipid Res* 2000; 41(4):605-12.
36. Polheim D, David K, Schult FM, mylie M B, Johnston J M. "Regulation of triglyceride biosynthesis in adipose and intestinal tissue. *J Lipod Res* 1973; 14: 415-21.
37. Mansbach II C M, Nevin P. "Intracellular movement of triacylglycerols in the intestine. *J Lipod Res* 1998; 39: 963-8.