Increased dietary triacylglycerol markedly enhances the ability of isolated rabbit enterocytes to secrete chylomicrons: an effect related to dietary fatty acid composition

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Abstract **Dietary fats are efficiently absorbed in the small intestine and transported into the blood via the lymph as chylomicrons, despite enormous variations in the amount and composition of the dietary lipid. The aim of the present study was to investigate how enterocytes respond to increased dietary fats of different composition. Rabbits were fed a low fat chow diet, and chow supplemented with sunflower oil (high n–6 polyunsaturated fatty acids), fish oil (high n–3 polyunsaturated fatty acids), or an oil mixture of a composition similar to that of the typical western diet. Feeding fat for 2 weeks markedly stimulated the ability of the isolated enterocytes to synthesize and secrete apolipoprotein B48, triacylglycerol, and cholesteryl ester (up to 18-, 50-, and 80-fold, respectively) in particles of chylomicron density. The magnitude of stimulation was sunflower oil > western diet lipid > fish oil. Single doses of lipid given 18 h prior to isolation of enterocytes stimulated chylomicron secretion by only 10% of that observed after 2 weeks of dietary supplementation. Enterocytes are replaced rapidly (half-life 1–2 days) by cells which move from the crypts to the tips of the villi, where absorption of nutrients takes place. Our observations suggest that dietary lipids modulate the function of enterocytes as they move from the crypts, so that the cells are 'turned-on' to lipid absorption. The results also show that diets of different fatty acid composition vary in their effects.**—Cartwright, I. J., and J. A. Higgins. **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: **1858–1866.**

Supplementary key words apoB-48 • triacylglycerol • cholesteryl ester • dietary fat • sunflower oil • fish-oil • western diet • fat absorption • small intestine

Absorption of dietary lipids involves intralumenal digestion, transfer of the products of digestion across the brush border of the enterocytes, resynthesis of the lipids, and assembly of chylomicrons, which are released into the lamina propria and move via the lymph into the circulation. Chylomicrons are light particles ($d < 1.000$ g/ml) which are heterogeneous in size (diameters 80–1000 nm) and consist of >90% neutral lipid, predominantly triacylglycerol (TAG) with some cholesteryl ester (CE), stabilized by a shell of amphipathic lipids, phospholipid and cholesterol, and protein (1). Apolipoprotein B-48 (apoB-48) is the major structural protein component and is essential for the formation and secretion of chylomicrons (1, 2). Electron microscopic studies have shown that the assembly of chylomicrons follows the classic secretary pathway; the lipid and protein components appear in the lumen of the endoplasmic reticulum of the enterocytes, from where they move to the Golgi lumen (3–6). Trans-Golgi vesicles then move to fuse with the lateral borders of the enterocytes releasing the chylomicrons outside the cell. The outline of events in the assembly of chylomicrons resembles that of very low density lipoproteins (VLDL), which transport endogenous lipids into the circulation from the liver. The major differences are that VLDL are considerably smaller than chylomicrons (40–80 nm in diameter), contain proportionally less neutral lipid, and that apoB-100 is the essential structural component of VLDL. ApoB-100 and apoB-48 are products of the same gene. However, in the intestine, the mRNA for apoB-100 is edited by a deaminase (apo-bec-1) which converts cytosine at position 6666 to uracil resulting in codon 2153 being converted to a stop codon. Translation of the edited message produces the truncated form, apoB-48 (7, 8). The significance of apoB editing is not known; although, as this occurs in the small intestine of all mammals, it appears likely that apoB-48 has a fundamental role in chylomicron assembly.

There has been considerable progress in elucidation of

Abbreviations: TAG, triacylglycerol; CE cholesterol ester; apo-B48, apolipoprotein B-48; DMEM. Dulbecco's modified Eagle's medium; VLDL, very low density lipoprotein; PUFA, polyunsaturated fatty acids.

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the molecular details of VLDL assembly in hepatic cells (9–11), however, very little is known about the intracellular and molecular details of chylomicron assembly and secretion in normal enterocytes. This is in part due to the lack of an appropriate cellular model for such studies (1). CaCo-2 cells have been used for a number of investigations (12, 13) However, these cells are not an ideal system as they secrete lipoprotein particles of the density of LDL/VLDL which contain apoB-100 as well as apoB-48. Nor is it possible to investigate the effects of diet on chylomicron assembly using cultured cells. In order to investigate chylomicron assembly, we have recently developed a method for the isolation and maintenance of viable rabbit enterocytes (14). The use of an animal model allows us to investigate the effect of diet on the characteristics and function of the enterocytes. We have used the rabbit because it is considered a good model for studies of lipoprotein metabolism and provides a good yield of enterocytes capable of secreting chylomicrons when provided with a physiological substrate (14).

The small intestine is subjected to large fluctuations in the amount and composition of dietary fats, which must be absorbed efficiently and assembled into chylomicrons. However, the way in which enterocytes respond to a dietary fat challenge and the factors regulating fat absorption are not understood. In the present study we have investigated the effect of manipulation of the TAG content and composition of the diet on the ability of the subsequently isolated enterocytes to secrete chylomicrons. Our observations suggest that increased dietary lipid over a 2-week period of time markedly increases the ability of the isolated enterocytes to secrete chylomicrons. This effect is related to the fatty acid composition of the diet.

MATERIALS AND METHODS

Materials

Dulbecco's modified Eagle's medium without methionine (DMEM) was from Gibco. [^{35}S]methionine and [^{3}H]oleate were from Amersham. High performance thin-layer chromatography silica gel plates (HPTLC) were from Cammag. PHM-Liposorb and protease inhibitor cocktail (Complete™) were from Calbiochem and Boehringer Mannheim, respectively. All other reagents were from Sigma or as described previously (14–21).

TABLE 2. Comparison of the fatty acid composition of the western diet with that of a typical UK diet

	Western Diet	MAFF Survey (mean value)
	%	%
Fat intake as calories	21	38
Saturated fatty acids	48.2	46.7
Monounsaturated fatty acids	28.9	35.0
Polyunsaturates	19.1	16.6

The values reported are from the MAFF Dietary and Nutritional Survey of British Adults (22). The % fatty acids are expressed as a % of the total fat.

Animals and diets

Dwarf lop rabbits (~6 months old, 2.56 ± 0.12 kg) bred in the University of Sheffield Field Laboratories were used for these studies. They were allowed free access to water and chow (2.5% fat w/w; equivalent to 7% of the dietary energy content, average intake 95 g/day) and were maintained on a 12-h light/dark cycle. Four dietary regimes were used: low fat chow, and chow supplemented with 5 ml/day of sunflower oil, fish oil, or a mixture of oils similar to that found in the typical Western diet (22). The fatty acid compositions of the diets and the contribution of each fatty acid to the energy intake are shown in **Table 1** and **Table 2**. Animals were gavaged each day, as described previously (23), with fish oil, sunflower oil, or the western diet oil mixture. The latter was formulated by melting and mixing lard (65.5%) with sunflower oil (17.25%) and triolein (17.25%). As described previously, to minimize oxidation of polyunsaturated fats (PUFA), oils were stored at 4°C in 10-ml syringes which were used for gavage and warmed to room temperature immediately before use (23). The western diet oil mixture was liquefied by warming the syringes to 37°C. Gavage was carried out by trained personnel in the Field Laboratories, who also monitored food intake and the health of the animals. The contribution of fat to energy in the chow diet was 7% and this increased to 21% in the fat-supplemented diets. Diets were administered daily for 2 weeks, with the last dose 24 h before the animals were killed. In some experiments the short term effect of each diet was determined by a single administration of the appropriate oil or oil mixture 18 h prior to preparation of isolated enterocytes.

Preparation of lipid/bile salt micelles

Lipid micelles of a composition similar to those found in the lumen of the small intestine (24) were prepared by sonication as described previously (14). The final concentrations of the micelle components in the incubation media were sodium cholate [0.14 mm]; sodium deoxycholate (0.15 mm); phosphatidylcholine (pal-

TABLE 1. Fatty acid composition of diets

	Chow	Sunflower Oil	Fish Oil	Western
	%	%	%	%
14:1 (myristoleic)	—	—	—	1.0 (0.21)
16:0 (palmitic)	19.3 (1.35)	6.5 (1.36)	20.8 (4.37)	36.8 (7.73)
16:1 (palmitoleic)	—	—	11.8 (2.48)	1.6 (0.34)
18:0 (stearic)	8.4 (0.59)	4.4 (0.92)	4.3 (0.90)	10.4 (2.18)
18:1 (oleic)	15.4 (1.08)	18.0 (3.80)	10.8 (2.27)	28.9 (6.07)
18:2 (linoleic)	55.5 (3.88)	69.7 (14.64)	2.5 (0.525)	18.6 (3.91)
18:3 (α-linoleic)	—	—	3.1 (0.65)	0.5 (0.11)
20:4 (arachidonic)	—	—	2.8 (0.59)	—
20:5 (eicosapentaenoic)	—	—	21.7 (4.56)	—
22:6 (docosahexaenoic)	—	—	15.7 (3.15)	—

The figure in parentheses indicates the % contribution of each fatty acid to the total calorie intake.

mitoleoyl) [0.17 mm]; oleic acid [0.22 mm] and monopalmitoylglycerol (0.19 mm).

Isolation and incubation of enterocytes

Enterocytes were isolated from the small intestine of rabbits and resuspended in oxygenated DMEM at 37°C, as described previously (14). The time of incubation in the final isolation buffer (solution C) was 15 min. This results in cell preparations which contain 80% of the alkaline phosphatase (marker for absorptive cells) with undetectable thymidine kinase (marker for crypt cells). The cell yield was similar from the small intestines of rabbits fed the four different diets. To measure the synthesis and secretion of apoB-48 the isolated cells (2 g in 3.6 ml of DMEM) were incubated with lipid/bile salt micelles (0.4 ml) and [^{35}S]methionine (100 μC). To measure the synthesis and secretion of TAG and CE, sufficient [^{3}H]oleate was added to the lipid/bile salt mixture prior to sonication to give a final concentration of 10 μC per 0.4 ml. Enterocytes were isolated for different times up to 90 min and pelleted by centrifugation at 800 *g* for 2 min. Chylomicrons were isolated by flotation from the incubation medium by centrifugation at 13,000 *g* for 20 min (14).

Analysis of apoB-48

Incorporation of [^{35}S]methionine into cellular and secreted apoB-48 and into total TCA-precipitable protein was determined as previously (14, 18–21, 25).

Extraction and analysis of lipids

Cellular and secreted lipids were extracted and separated by HPTLC as previously (14, 19, 25). Quantitation of ^{3}H radioactivity in TAG and CE was performed by scintillation counting. TAG mass was determined by laser densitometry (14, 19, 25).

RESULTS

Isolated enterocytes synthesize and secrete apoB-48 in particles of the density of chylomicrons

Isolated enterocytes synthesized apoB-48 from [^{35}S]methionine at a steady rate for the 90-min incubation period used (**Fig. 1A**). Secretion of radiolabeled apoB-48 was low

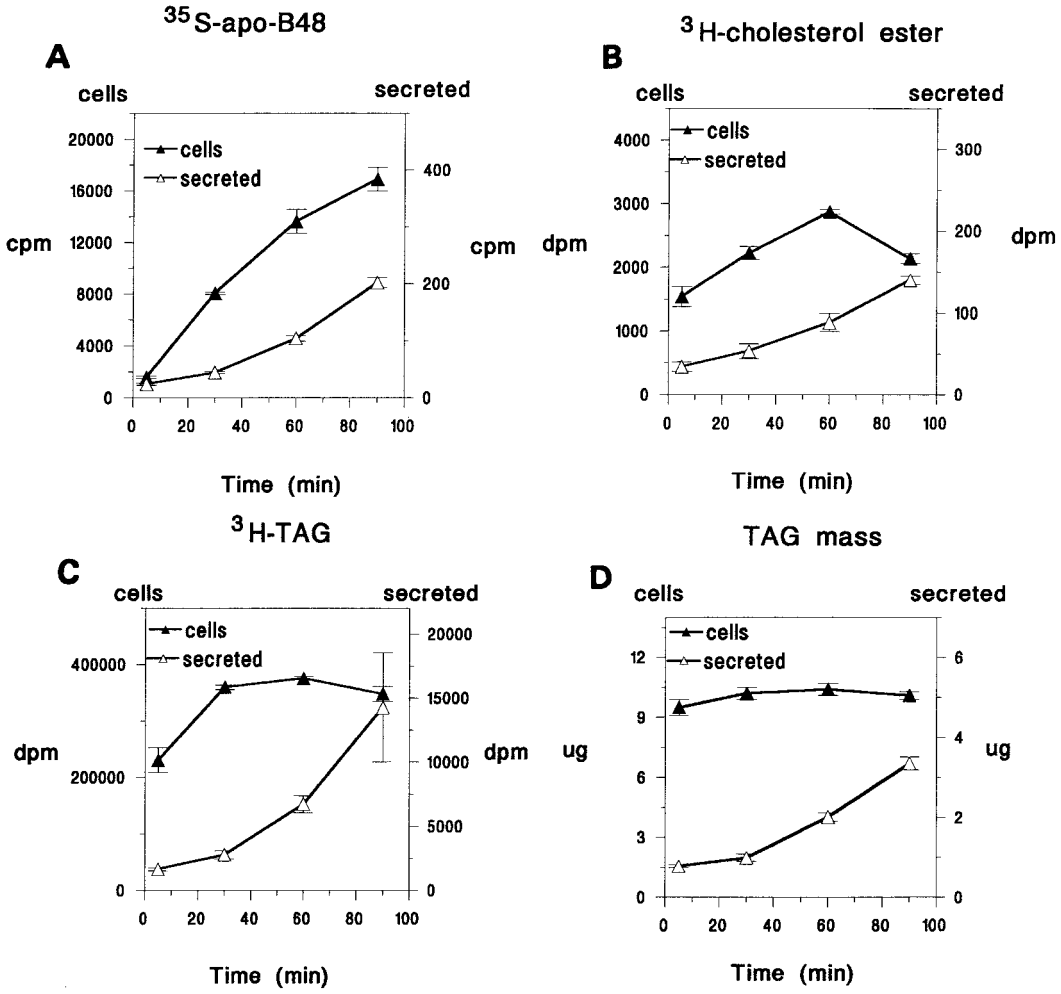

Fig. 1. Synthesis and secretion of chylomicron components by isolated rabbit enterocytes. Isolated enterocytes from chow-fed rabbits were incubated with [^{35}S]methionine or [^{3}H]oleate and micelles as described in Methods. At the end of each incubation period, the cells were pelletted by centrifugation and the incorporation of [^{35}S] methionine or [^{3}H]oleate into cellular and secreted (chylomicron) apoB-48 or lipids, respectively, was determined: the mass of TAG was also measured as described in Methods. A: Newly synthesized apoB-48; B: Newly synthesized CE; C: newly synthesized TAG; and D: mass of TAG. Results plotted are the mean of assays on three separate enterocyte preparations ± standard deviation.

for the first 30 min and increased during the remaining 60-min incubation. Isolated enterocytes also synthesized and secreted TAG and CE from [^{3}H]oleate (Fig. 1B–D). Synthesis of the radiolabeled lipids reached a peak in the cells after about 30–60 min incubation and secretion followed the same pattern as apoB-48. As shown previously (14), secretion was dependent on addition of micelles to the incubation medium and >90% of the secreted radiolabeled apoB-48 and lipids was recovered in the chylomicron fraction floated from the incubation medium by centrifugation at 13,000 *g* for 20 min (data not shown).

Only 1–2% of the newly synthesized apoB-48 and 5–7% of the newly synthesized lipid was secreted after 90 min incubation, indicating that synthesis of these chylomicron components is not a limiting factor in secretion. However, the mass of TAG in the enterocytes was relatively constant at ~9 μg per mg of cell protein, while secretion reached ~4 μg per mg cell protein. The isolated enterocytes therefore synthesize and secrete TAG efficiently. However, the newly synthesized TAG does not equilibrate with the secreted pool and a large fraction is retained in the enterocytes at least during the relatively short incubation periods used in our protocols.

Enterocytes isolated from rabbits fed fat-enriched diets for 2 weeks have a markedly increased ability to synthesize and secrete chylomicrons

Feeding fat stimulated synthesis and secretion of apoB-48, TAG, and CE by isolated enterocytes, expressed per mg of cell protein. (**Fig. 2**). As in the case of the enterocytes from animals fed low fat chow, >90% of the secreted apoB-48 and lipid were recovered in the chylomicron fraction. In all cases, feeding sunflower oil had the greatest effect and fish oil the least effect, with the western diet oil mixture intermediate. Synthesis of apoB-48 in cells from sunflower oil-fed animals was increased by 10-fold and secretion of newly synthesized apoB was increased by 18-fold compared with enterocytes from chow-fed animals. The incorporation of [^{35}S]methionine into total TCA-insoluble cellular and secreted protein was similar in enterocyte preparations from animals in all four dietary groups (data not shown). Therefore, the stimulation of apoB-48 synthesis and secretion is not a consequence of generalized effects of fat feeding on protein synthesis and secretion. TAG and CE synthesis were increased ~7-fold and ~10-fold in cells from sunflower oil-fed animals and secretion of radiolabeled TAG and CE was increased 53-fold and 84-fold, respectively. A greater proportion of the newly synthesized lipid was secreted by enterocytes from fat-fed animals Thus, ~30% of the total radiolabel incorporated into cellular TAG and CE of enterocytes from sunflower oil-fed rabbits appeared in the secreted chylomicrons after 120 min compared with ~7% of the total newly synthesized TAG and CE secreted by cells from chow-fed controls. From the data in Fig. 2B and D it can be calculated that the specific activity of the TAG secreted by enterocytes from chow-fed rabbits is approximately 1/10 that of the cellular TAG. In the enterocytes from fat-fed rabbits, the specific activity of the cellular TAG increased up to ~3-fold, with sunflower oil > western diet > fish oil. Fat feeding therefore stimulates synthesis of TAG from [^{3}H]oleate provided in micelles. In addition, the specific activity of the TAG secreted by enterocytes from fat-fed rabbits was >10-fold greater than that secreted by enterocytes from chow-fed rabbits, although the specfic activity of the cellular TAG was always greater than that of the secreted TAG.

A single acute dose of dietary lipid has only a small effect on the ability of isolated enterocytes to synthesize and secrete chylomicrons

The enterocytes of the small intestine are replaced rapidly. About 70% of the uptake of lipids is carried out by enterocytes at the top one-third of the villi, when the cells are about 30 h old (26). The effect of the fat-enriched diets may thus be on the biochemical function of the cells as they move from crypt to villus, or an acute effect on the mature enterocytes. To investigate this, a single dose of each oil was given to animals 18 h prior to cell isolation. Under these conditions, there was no significant difference in the mass of cellular TAG or secreted TAG in enterocytes from all four dietary groups (**Fig. 3**). Compared with the 2-week dietary treatment, only relatively small stimulations were observed in the synthesis and secretion of apoB-48, TAG, and CE by isolated enterocytes with sunflower oil > western diet > fish oil. Synthesis of apoB-48, TAG, and CE was increased approximately 2-fold, 1.4-fold, and 2-fold, respectively by feeding sunflower oil with western diet oil and fish oil having smaller effects (Fig. 3). Secretion of apoB and TAG was increased approximately 2-fold by the sunflower oil-fed enterocytes and secretion of CE by 6-fold. These effects were <10% of those observed in enterocytes from animals fed increased fat for 2 weeks and can be accounted for by changes in the biochemical activity of part of the cell population during the 18 h after the fat dose.

DISCUSSION

Isolated rabbit enterocytes synthesized and secreted lipoprotein particles of the density of chylomicrons when presented with a substrate consisting of micelles of physiological composition. Feeding an increased fat diet markedly stimulated the capacity of isolated enterocytes to synthesize and secrete apoB-48, TAG, and CE expressed per mg of cell protein. Identical conditions were used for isolation of cells in all dietary experiments and the yields and characteristics of the cells isolated were similar. We have also examined intestinal sections from the rabbits and there was no marked change in the morphology of the villi in the fat-fed compared with chow-fed animals. Thomson et al. (27) have shown that feeding isocaloric diets rich in saturated or unsaturated fat to rats has no effect on the crypt cell production rate compared with a control low fat diet, but does increase the height of the villi and the enterocyte migration rate by approximately 30% for either diet. This would not be sufficient to account for the marked effects of dietary lip-

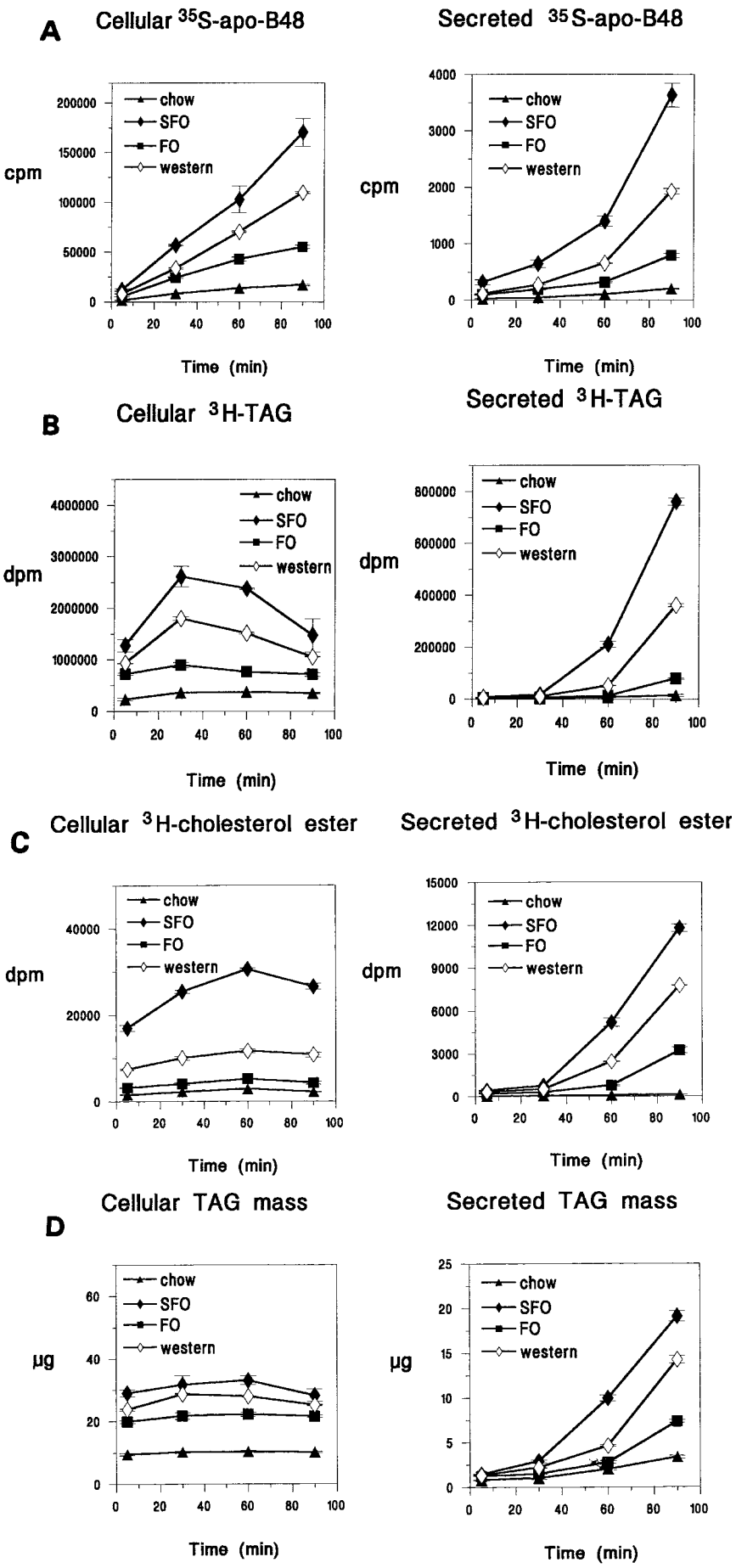

Fig. 2. Effect of dietary fat supplementation for 2 weeks on the synthesis and secretion of apoB-48, TAG, and cholesteryl ester by isolated enterocytes. Isolated enterocytes from rabbits fed chow or chow supplemented with different oils for 2 weeks were incubated with [^{35}S]methionine and micelles or with micelles containing [^{3}H]oleate, as described in Methods. At the end of each incubation period, the cells were pelletted by centrifugation and the incorporation of A: [^{35}S]methionine into cellular and secreted (chylomicron) apoB; B: ^{3}H into celular and secreted TAG; C: ^{3}H into cellular and secreted CE; and D: the mass of cellular and secreted TAG were determined. Results plotted are the mean of assays on three separate enterocyte preparations ± standard deviation; FO, fish oil; SFO, sunflower oil; western, oil of composition of western diet. In comparison with the enterocytes from chow-fed rabbits, there was a significant change ($P < 0.002$), after 120 min incubation, in the cellular and secreted apoB-48, TAG, and CE in all enterocyte preparations from rabbits fed fat-enriched diets.

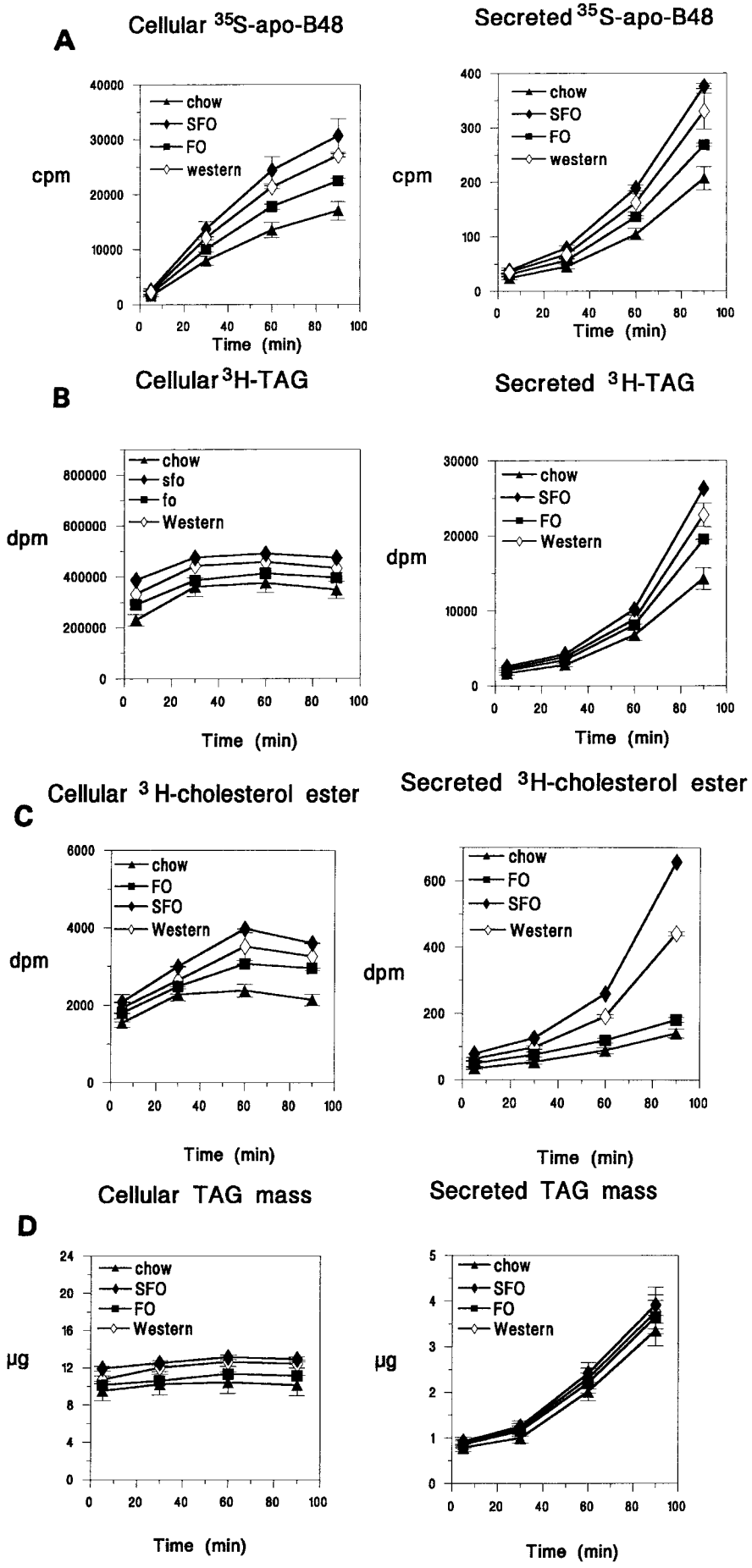

Fig. 3. Effect of a single dietary fat supplement on the synthesis and secretion of apoB-48, TAG, and cholesteryl ester by isolated enterocytes. Isolated enterocytes, from rabbits fed a single dose of different oils 18 h before they were killed, were incubated with [^{35}S]methionine and micelles or with micelles containing [^{3}H]oleate, as described in Methods. At the end of each incubation period, the cells were pelletted by centrifugation and the incorporation of A: [^{35}S]methionine into cellular and secreted (chylomicron) apoB; B: ^{3}H into cellular and secreted TAG; C: ^{3}H into cellular and secreted cholesteryl ester; and D: the mass of cellular and secreted TAG were determined. Results plotted are the mean of assays on three separate enterocyte preparations ± standard deviation; FO, fish oil; SFO, sunflower oil; western, oil of composition of western diet. The changes in the synthesis of cellular and secreted apoB, TAG, and CE were not significantly different ($P \geq 0.1$) in enterocytes from fat-fed rabbits compared with enterocytes from chow-fed rabbits, with the exception of cellular and secreted CE in enterocytes from sunflower oil-fed rabbits ($P < 0.05$).

ids observed in the present study. Nor can these observed effects on isolated enterocytes be accounted for by physiological changes, such as increased lymph flow, which has been reported to occur in response to dietary changes (5, 26). Only a small stimulation of chylomicron secretion was observed in enterocytes 18 h after feeding a single dose of lipid. Therefore, the most likely explanation for our observations is that the chronic feeding of a fat-enriched diet modulates the biochemical function of the enterocytes, so that the cells become adapted to chylomicron assembly.

For these studies we supplemented the control chow diet with fat of three different compositions. The sunflower oil- and fish oil-supplemented diets were enriched in n−6 and n−3 PUFA, respectively. However, because the fatty acid composition of these diets is somewhat extreme compared with the normal human diet, we also used a western diet containing mixed fat of a composition similar to that of the diet of the UK population (22). As in previous studies, the oils were given to the animals by daily gavage (23). This was because rabbits are reluctant to eat high-fat chow. However, gavage does have the advantage that the amount of fat fed is controlled, no oxidation occurs due to exposure to air, and the single dose mimics a high fat meal. The contribution of fat to the energy content of the diet was 7% for the chow and ~21% for the fat enriched diets. This is less that the fat content of the average western diet which is 35–40% (22). However, as marked effects were seen with ~21%-fat diets, we chose to gave a smaller volume of oils, which was well tolerated by the animals.

The stimulatory effect of the fat-enriched diets was sunflower oil > western diet > fish oil for synthesis and secretion of apoB-48, TAG, and CE. There was a linear correlation ($r > 0.9$) between the increased secretion of apoB-48 and newly synthesized TAG, CE, or TAG mass, suggesting that the whole process of chylomicron assembly and secretion was stimulated (**Fig. 4**). The proportion of lipid in chylomicrons also increased, suggesting that larger chylomicrons were synthesized as secretion was stimulated, as reported by others (28). The effect of the diets was not simply due to an increase in fat content but differed with fatty acid composition. However, there was no simple relationship between the contribution of any single fatty acid to dietary energy and secretion. When those fatty acids which were present in all four diets were compared, an increase in the amount of saturated fatty acids (palmitic and stearic acids) and oleic acid correlated ($r > 0.9$) with increased secretion by enterocytes prepared from animals fed chow, the western diet and fish oil, but not sunflower oil (**Fig. 5**). An increase in linoleic acid (n−6) correlated ($r > 0.9$) with increased secretion by enterocytes from rabbits fed the three fat-enriched diets but not from chow-fed rabbits (Fig. 5). The overall effect of the mixed fatty acid diets is therefore probably a composite of different effects of different fatty acids. In the present study, we chose to use dietary fatty acid compositions that fall broadly within the limits of normal human nutrition. However, to determine whether individual fatty acids have specific effects, it will be necessary to carry out experiments in which the content of single fatty acid is varied in isocaloric diets.

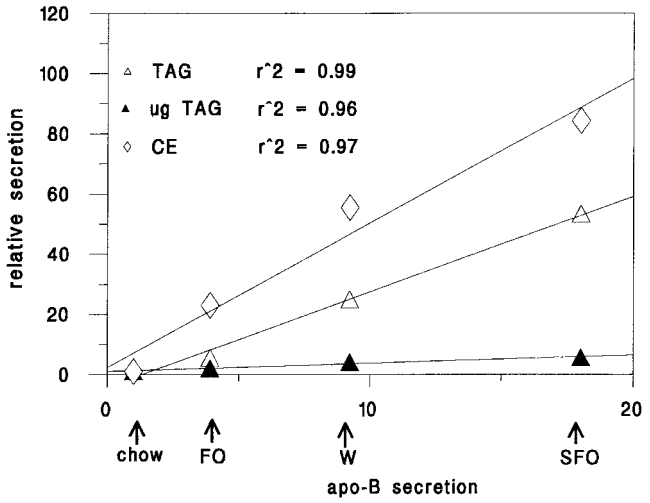

Fig. 4. Correlation of apoB secretion with secretion of newly synthesized TAG, TAG mass, and newly synthesized cholesteryl ester by isolated enterocytes from rabbits fed different dietary fats. The relationships between the stimulation of secretion of apoB-48 and TAG, CE and TAG mass by different dietary fat supplementations are plotted using the data from experiments shown in Figs. 2–5. The arrows indicate the data for each diet chow; FO, fish oil-enriched diet; SFO, sunflower oil-enriched diet; W, western diet.

We have previously reported that the specific activity of the TAG secreted by enterocytes from chow-fed rabbits is considerably lower than the specific activity of the cellular TAG (14). This observation was repeated in this experiment. Feeding fat increased the specific activity of the cellular TAG by ~3-fold, and the specific activity of the secreted TAG by >10-fold compared with enterocytes from chow-fed rabbits. These observations agree with our earlier conclusions that there is more than one intracellular pool of TAG in enterocytes and that TAG newly synthesized from [^{3}H]oleate in micelles is preferentially retained in the cells (14). However, fat feeding changes the balance between TAG pools so that a greater proportion of the newly synthesized TAG is secreted. This effect is related to the composition of the dietary fat with sunflower oil having the greatest effect.

In contrast to observations on hepatocytes in which VLDL secretion was inhibited by incubation with n−3 PUFA or by feeding fish oil (21, 23, 29, 30), chylomicron secretion was stimulated in enterocytes from rabbits fed fish oil for 2 weeks and was not inhibited by short term treatment. It may be that n−3 PUFA do not affect chylomicron assembly in the same way as VLDL assembly. Alternatively, the stimulatory effect of an increased fat diet may override the inhibitory effect of fish oils on chylomicron assembly. Fat-enriched diets had a far greater effect on chylomicron assembly than on VLDL assembly; for example, apoB-48 secretion by enterocytes was increased >18-fold by feeding sunflower oils compared with an increase of 2- to 3-fold in apoB-100 secretion by isolated hepatocytes (21).

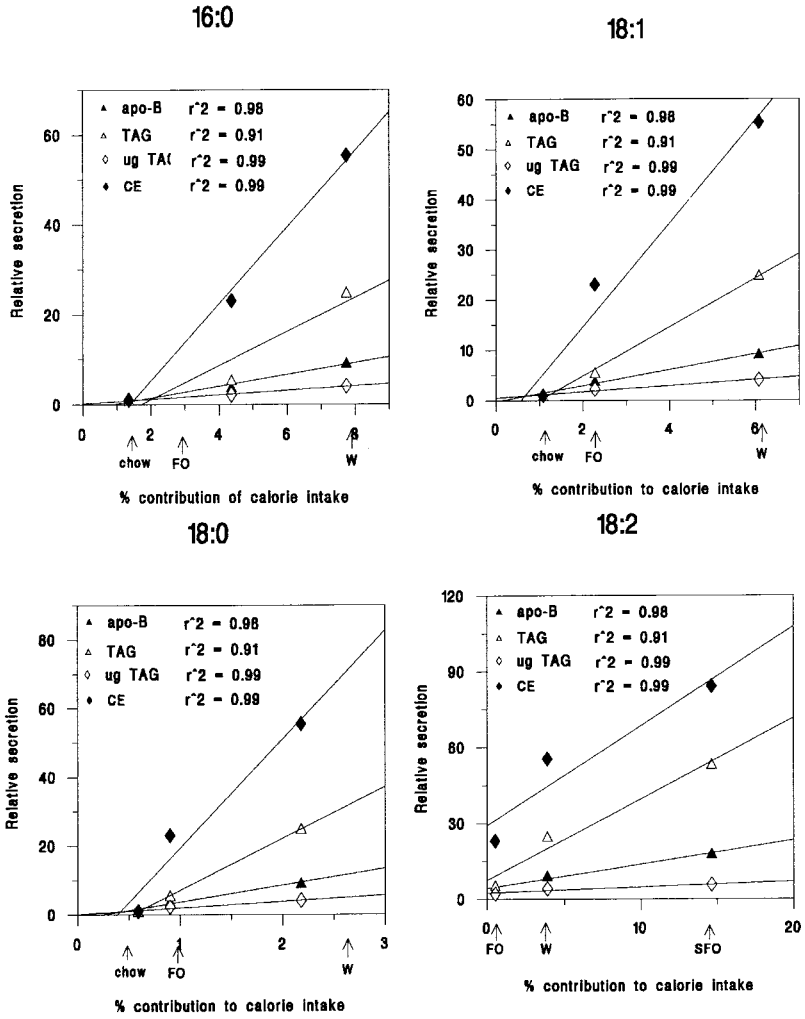

Fig. 5. Correlation of the contribution of different fatty acids to dietary energy content and the relative secretion of chylomicron components by isolated enterocytes. The relationship between the relative contributions of different fatty acid components to the calorie intake of different diets is plotted against the relative secretion of newly synthesized apoB-48, TAG, and CE and the mass of TAG. The data are taken from the experiments shown in Figs. 2–5. The arrows indicate the data for each diet chow; FO, fish oil-enriched diet; SFO, sunflower oil-enriched diet; W, western diet.

The epithelium of the small intestine has an extremely rapid turnover. Stem cells at the base of the crypts divide and move up the crypts to the villi to replace the epithelium which is sloughed off into the lumen (1, 26, 27). As the cells travel up the crypt they differentiate and those cells at the top one-third of the villi are most active in absorption of nutrients. The population of absorptive cells is replaced after ~3 days (26, 27). Thus, the small intestinal epithelium has a unique capacity to adapt to dietary change over a relatively short time period. Our observations show that increased dietary fat during maturation causes adaptation of the enterocytes so that they have an increased capacity to absorb lipid and secrete chylomicrons. The magnitude of stimulation is influenced by the fatty acid composition of the diet.

Although, apoB-48 is an essential structural protein of chylomicrons, its synthesis is not rate-limiting in chylomicron secretion (31, 32). ApoB-48 is synthesized in excess and the excess protein is degraded intracellularly (14). However, there are many other biochemical and cellular steps at which chylomicron secretion may be altered by dietary lipids. These include *i*) transport of lipid across the brush border, *ii*) translocation of apoB-48 into the secretory pathway, *iii*) synthesis of chylomicron lipids and translocation of these into the lumen of the secretory compartment, and *iv*) movement and assembly of apoB-48 and lipids within the vesicular elements of the endoplasmic reticulum/Golgi compartment. The mechanism by which dietary fatty acids alter the ability of enterocytes to absorb lipid may thus be to activate or to increase gene expression of one or more of the proteins involved in these processes during differentiation. A number of studies have suggested that different fatty acids have differential effects on gene expression (33, 34). Candidate proteins include fatty acid binding proteins involved in movement of fatty acids across the brush-border membrane and through the cytosol to the endoplasmic reticulum (5, 35), enzymes of lipid synthesis in the endoplasmic reticulum membrane (e.g., monoacylglycerol acyl-CoA acyltransferase, diacylglycerol acyl-CoA acyltransferase, and acyl-CoA cholesterol acyltransferase) (1, 5), proteins implicated in apoB-48 translocation into the secretory pathway (e.g., microsomal triglyceride transfer protein and BiP) (1, 5), and putative factors involved in intracellular vesicular transit of apoB-48 and lipids (36–38). Isolated enterocytes will provide a useful model system for determining which of these proteins is involved in adaptation to dietary changes.

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