The effects of fatty acids on apolipoprotein B secretion by human hepatoma cells (HEP G2)

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Abstract

We have investigated the effect of fatty acids on the rate of apolipoprotein B (apo B) secretion by human hepatoma cells (Hep G2). When Hep G2 cells were maintained in tissue culture flasks oleic acid up to 0.4 mM increased apo B secretion in a dose-dependent manner, whereas increases in triacylglycerol (TG) were smaller and dose dependency was less evident. In the absence of oleic acid, apo B accumulating in the tissue culture medium was predominantly in lipoproteins of higher density than very low density lipoproteins (VLDL). However, when the rate of secretion was stimulated with oleic acid the apo B-containing lipoproteins became lower in density. We postulated that there was a high rate of lipolysis of newly secreted VLDL by Hep G2 cells, which would account both for the relatively smaller effect of oleic acid on TG as opposed to apo B accumulating in the culture medium and the predominance of apo B in lipoproteins of a higher density than VLDL, which became less evident when VLDL secretory rates were stimulated by oleic acid. To test this hypothesis, cultured Hep G2 cells were transferred to columns containing Cytodex beads, permitting their continuous perfusion with culture medium so that newly secreted VLDL did not remain in contact with the cells. Apo B recovered from the perfusate was largely in VLDL range lipoproteins and the TG measured in the perfusate indicated that the true secretory rate of TG-rich lipoproteins was substantially higher than had been reflected by TG accumulating in culture medium left in contact with cells. Apo B measured in the culture medium of Hep G2 cells may thus be a better reflection of VLDL secretion, even though it is contained in higher density lipoproteins due to removal of TG by lipolysis. The effects of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) on apo B (apo B) secretion by Hep G2 cells maintained in tissue culture flasks were next investigated. SFA (0.4 mM), with the exception of stearic acid (C18:0), increased apo B secretion. Lauric acid (C12:0) increased apo B secretion by 32%, myristic acid (C14:0) by 41% (P < 0.005), palmitic acid (C16:0) by 154% (P < 0.025), and arachidic acid (C20:0) by 186% (P < 0.005). The effect of MUFA (0.4 mM) was to increase apo B secretion, oleic acid (C18:1) by 239% (P < 0.0005) and palmitoleic acid (C16: 1) by 125% (P < 0.005). Of the PUFA investigated, linolenic acid (C18:3) (0.4 mM) did not have any significant effect on apo B secretion, whereas linoleic acid (C18:2) (0.4mM) arachidonic acid (C20:4) (0.1 mM) and eicosapentaenoic acid (C20:5) (0.1 mM) caused significant increases of 164, 171 and 171%, respectively (P < 0.005). The fatty acids studied increased intracellular TG and cholesteryl ester concentrations to varying extents. The increase in intracellular TG produced by the different fatty acids correlated with the rate of apo B secretion (r = 0.6; P < 0.05). In this human hepatoma cell line, with the exception of the saturated fatty acids, the rate of secretion of apo B-containing lipoproteins does not follow the same pattern as changes in circulating low density lipoprotein (LDL) concentrations reported with dietary manipulation in man. If our findings reflect the in vivo situation, we suggest that whilst the dietary effects of SFA on serum LDL may in part be determined by the hepatic apo B secretory rate, the effects of MUFA and PUFA must be largely mediated through a catabolic effect rather than an effect on hepatic secretion. The marked increase in apo B secretion with the more highly polyunsaturated fatty acids, such as eicosapentaenoic acid, may also explain why they do not lower circulating LDL, despite reports of their apparently favourable effect on LDL-receptor mediated clearance. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

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1. Introduction

Dietary fatty acids play an important role in determining plasma total cholesterol (TC) concentrations in humans. Saturated fatty acids (SFA) of chain length C12–C16 have been demonstrated to be the most hypercholesterolaemia-inducing fatty acids [1]. In contrast, polyunsaturated fatty acids (PUFA), such as linoleic acid, and monounsaturated fatty acids (MUFA), such as oleic acid, when substituted for SFA in the diet, lower plasma TC concentrations [2,3] and this forms the basis of current dietary recommendations to reduce plasma TC and low density lipoprotein-cholesterol (LDL-C) concentrations [4]. Highly polyunsaturated fatty acids such as eicosapentaenoic acid and docosahexaenoic acid, although they do not decrease serum cholesterol, are effective in lowering serum triacylglycerol (TG) concentrations [5].

It has been well documented that Hep G2 cells synthesise and secrete lipoproteins which contain the apolipoproteins B-100, A-I, A-II, A-IV, B, C-I, C-II, C-III and E [6–10]. Apolipoprotein B-100 (apo B) is the sole apo B species secreted by the human liver [11], and is also the apo B species synthesised and secreted by the Hep G2 cell line [7]. Hep G2 cells maintain many of the morphological and biochemical characteristics of normal hepatocytes [7,10,12,13]. They have, however, been criticised as a model of the hepatic secretion of apo B-containing lipoproteins, because unlike, for example rat hepatocytes, the apo B which accumulates in the culture medium of Hep G2 cells is not principally in very low density lipoprotein (VLDL), but is also substantially present in the low density lipoprotein (LDL) and even denser lipoprotein particles. We have previously suggested, however, that the apo B-containing VLDL secreted by Hep G2 cells is rapidly converted to LDL, because of the high activity of extracellular hepatic lipase produced by Hep G2 cells [14]. Thus the apo B accumulating in the medium reflects the rate of VLDL secretion, although the concentration of TG in the medium does not necessarily reflect its rate of secretion because of lipolysis. In the present investigation we examined this hypothesis further in a cell culture system which allows the rapid removal of newly secreted lipoproteins from further contact with Hep G2 cells. This part of the investigation confirmed our hypothesis. We therefore progressed to study the effects of individual saturated, monounsaturated and polyunsaturated fatty acids on apo B secretion, using a conventional Hep G2 cell culture system, which may provide an in vitro model to assist in explaining the results of dietary experiments with foods of different fatty acid composition, which cannot readily distinguish effects on the secretion of apo B-containing lipoproteins from those on their catabolism.

2. Methods

2.1. Materials

Dulbecco’s Modified Eagle’s Medium (DMEM) containing 25 mM glucose, foetal calf serum (FCS), penicillin/streptomycin solution, trypsin/EDTA solution (10 ×) and nystatin were purchased from Life Technologies Ltd (Paisley, UK).

Fatty acid free-fraction V bovine serum albumin (BSA), saturated fatty acids, monounsaturated and polyunsaturated fatty acids, beta-hydroxybutyrate kits and lactate dehydrogenase kits were all supplied by the Sigma Chemical Co. (London, UK).

Tissue culture flasks, 25 cm², were purchased from Bibby, (Nottingham, UK). Pierce BCA protein reagent was supplied by Pierce Chemical Co. (Pierce & Wariner, Chester, UK). Horse anti-sheep IgG decanting Solution 2 was from Pharmacia LKB (Milton Keynes, UK). Monoclonal antibody to apolipoprotein B was obtained from Biogenesis Ltd. (Bournemouth, UK). The non-esterified fatty acid kit was supplied by the WAKO Chemical Co. (Alpha Laboratories, Hants, UK).

TG and cholesterol reagent (GPO-PAP and CHOD-PAP) were purchased from Biostat Ltd. (Stockport, UK) and free cholesterol reagent was from Boehringer Mannheim BCL (Lewes, Sussex, UK).

Amicon centriflo membrane cones type CF25, conical supports CS1A, and centrifuge tubes CTI, were supplied by Amicon Ltd. (Storehouse, Gloucestershire, UK).

2.2. Cell culture

Human hepatoblastoma cells, Hep G2, were supplied from the European Collection of Animal Cell Culture [15]. Hep G2 cells were cultured in DMEM containing 25 mM glucose, penicillin (1000 U/ml), streptomycin (100 µg/ml) and 10% FCS (v/v) at 37°C in an atmosphere of 95% air/5% CO₂, with one medium change, for 7 days prior to an experiment, after which time they were confluent, as observed by light microscopy (4 × 10⁵ cells per flask), viability was 96 ± 0.5% as assessed by Trypan blue exclusion. Hep G2 cells were incubated in serum free medium for 24 h prior to any experiment, after which time the cells were washed twice with sterile phosphate buffered saline (PBS) and then the appropriate fatty acid (FA) complexed to 1% BSA (w/v) by drop-wise addition of FA dissolved in ethanol to a solution of BSA with continuous stirring, was added in FCS-free medium for a further 24 h. In all experiments 1% BSA was used as a control, and the concentration of FA that was added to the cells was 0.4 mM (molar ratio BSA:FA 2.7:1), except with arachidonic acid and eicosapentaenoic acid when 0.1 mM was added (molar
ratio 10.8:1). After incubation, medium was removed for analysis and the cells washed with phosphate buffered saline (PBS) pH 7.2 and harvested using a rubber policeman. The cells in PBS containing 1% triton X-100 were then disrupted using a Kontes micro ultrasonic cell disrupter. The average cell protein per incubation was between 0.5 and 0.6 mg.

2.3. Cell culture on perfused beads

2.3.1. Preparation of Cytodex 2 microcarrier beads

A total of 1 g of Cytodex 2 beads were added to 50 ml PBS in a glass bottle and incubated over-night at 37°C to hydrate the beads. They were then autoclaved, washed in PBS and allowed to settle for 10 min. The PBS was removed and to 5 ml of beads an equal volume of DMEM was added to make 50% slurry [16].

2.3.2. Adhering Hep G2 cells to the microcarrier beads

Two confluent flasks of Hep G2 cells were trypsinised, centrifuged and resuspended in DMEM. These cells (4×10^6) and the slurry of Cytodex beads were added to an autoclaved 125 ml Techne stirrer bottle (Phillip-Harris, Hull, UK) and the volume made to 50 ml with DMEM. This was placed on a hot plate magnetic stirrer (35–40°C) to stir continuously at approximately 40 rpm. After 3 h the volume was increased to 100 ml with DMEM and allowed to continue stirring over-night. Following this, the beads were allowed to settle and the medium removed and replaced with 10 ml of DMEM.

2.3.3. Cell perfusion

The beads were poured into a 10×0.5 cm^2 chromatography column. The column was perfused with fresh sterile medium, kept at 37°C in a sterilised container. Medium was passed over the Hep G2 cells at a constant rate of 6 ml/h and collected in 2 ml fractions over a period of 24 h. These fractions were used for lipoprotein fractionation by discontinuous gradient ultracentrifugation (DGU) and subsequent measurement of protein apo B and TG concentrations.

2.4. Lipid and protein measurements

Protein was determined in cell homogenates and culture media using the Pierce BCA protein reagent (Pierce and Warriher, Chester, UK). TG, free cholesterol (FC) and total cholesterol (TC) concentrations were measured using enzymic methods (GPO-PAP and CHOD-PAP) adapted and standardised for use in cell culture experiments as previously described by us [14]. Recovery experiments were carried out to compare cell homogenates with chloroform/methanol (2:1 v/v) dried lipid extracts, and the recoveries for TG, FC, and TC were 82.5±5.0%, 87.5±3.0%, and 90±4.5%, respectively. Cholesteryl ester (CE) was calculated by subtracting the FC from the TC measured in the cells.

2.5. Discontinuous density gradient ultracentrifugation (DGU)

A volume of 10 ml of medium in the flask experiments or a 2 ml fraction in the perfusion experiments were concentrated to 1 ml in Amicon Cones and lipoproteins separated by DGU as described previously [17] to produce fractions of Sf 60–400, 20–60, 12–20, 0–12 and < 0.

2.6. Immunoradiometric assay (IRMA) of apolipoprotein B

Apolipoprotein B secreted into the culture medium from Hep G2 cells was measured using an immunoradiometric assay (IRMA) (sensitivity 6 ng/ml) as previously described in detail [14] using 100 μl of sample and standard in the assay.

2.7. Measurement of non-esterified fatty acids (NEFA)

To ensure that all the FA had been complexed to the BSA, serum-free medium containing 1% BSA and fatty acids at the concentration used in the experiments, but which had not been exposed to Hep G2 cells, was concentrated 10-fold using Amicon centriflo cones. The cones have a filter with a molecular weight cut-off of 30 000 Da, therefore the BSA is retained within the cone and the medium which has passed through the cone will contain any non-BSA bound FA. The NEFA concentrations in a 50 μl sample of medium which had passed through the cone was determined using the WAKO NEFA-C kit according to the manufacturer’s instructions. Three standards (0.5, 1.0 and 1.97 mEq/l) were assayed in parallel and used to construct a standard curve.

2.8. Measurement of beta-hydroxybutyrate (β-HBA)

Beta-Hydroxybutyrate was measured in the medium from Hep G2 cells using a commercial method (Sigma Chemical Co) according to the manufacturer’s instructions.

2.9. Measurement of cytotoxicity

Lactate dehydrogenase (LDH) was measured using a commercial method (Sigma Chemical Co) in the medium from Hep G2 cells to ensure that the FA added to the cells did not cause cytotoxicity.
2.10. Statistical analysis

Differences between results were sought using a Student’s t-test and correlations between data sets were calculated using the Pearson’s correlation coefficient.

3. Results

In initial experiments carried out to determine the optimal concentration of FA stimulating VLDL secretion apo B was measured in the medium after 24 h incubation with increasing concentrations of FA. With all FAs there was progressive increase in apo B until at least 0.4 mM. The example of oleic acid is shown in Fig. 1. FA concentration of 0.4 mM is within the range encountered in the hepatic circulation [18]. At concentrations greater than 0.5 mM some of the FA became cytotoxic at the concentration of albumin used in our experiments. The exceptions were arachidonic acid and eicosapentaenoic acid, which caused Hep G2 cells to die at lower concentration. They were therefore studied at 0.1 mM. Medium TG concentration, unlike apo B, did not increase with increasing oleic acid and remained relatively constant over 24 h (Fig. 1).

When the medium from Hep G2 cells cultured in the presence and absence of 0.4 mM oleic acid was subjected to discontinuous gradient ultracentrifugation, the distribution of apo B between the density subfractions showed a higher proportion of apo B present in the less dense lipoprotein fractions than in medium not containing oleic acid in which Hep G2 cells had been cultured (Fig. 2). This could be interpreted as indicating that larger apo B-containing lipoprotein particles are secreted when oleic acid is present as a substrate. However, given the much higher rate of apo B secretion by cells grown in oleic acid-containing media (Fig. 1), this seemed unlikely to be the whole explanation for the phenomenon. We postulated that apo B was initially secreted by Hep G2 cells in VLDL both when oleic acid was present and absent, but that when oleic acid was present VLDL production was higher and the VLDL substrate in the culture medium exceeded the capacity of hepatic lipase present on the surface of hepatocytes to remove its TG and phospholipid, so that the proportion of less dense lipoproteins remaining in the culture medium was greater than when VLDL secretory rates were lower in the absence of oleic acid. In order to test further this hypothesis, we cultured the same number of hepatocytes (4 x 10^6) on Cytodex beads in a column which was perfused with culture medium at a rate of 6 ml/h so that newly secreted lipoproteins were not left in contact with cells. (Hepatic lipase is bound to glycosaminoglycans adherent to the cell surface). Under these conditions, 216.7 ± 41.7 µmol/24 h of TG was recovered in the perfusate, whereas cells grown in flasks yielded only 20.0 ± 4.2 µmol/24 h. The concentration of apo B in the perfusate was below the detection limit of our assay. However, when the perfusate was subjected to DGU after concentration twofold apo B could be measured in the lipoprotein fractions. This experiment (Fig. 3) revealed that apo B was largely present in the

Fig. 1. Apolipoprotein B (Apo B) and triacylglycerol (TG) accumulation in the medium from Hep G2 cells after incubation with increasing concentrations of oleic acid. Hep G2 cells were incubated with increasing concentrations of oleic acid, and the rate of accumulation of apo B (■) and TG (●) was measured over 24 h. Values are Mean ± SE of nine experiments. Significantly different compared to no oleic acid addition, ** P < 0.05, *** P < 0.005.
VLDL density range (Sf60–400 and 20–60). We have previously demonstrated that high lipase activity is expressed by Hep G2 cells [14]. The heparin-releasable lipase activity in our experiments was 8.82 ± 1.42 μmol/min per mg cell protein (n = 9) (mean ± SD) and was not significantly affected by the presence of any of the FA’s studied (results not shown). The concentration of apo B accumulating in the culture medium over a given time period in non-perfusion experiments is thus a better indication of VLDL secretion than TG which is rapidly hydrolysed.

3.1. Effects of fatty acids on apo B secretion, and intracellular cholesteryl ester and triacylglycerol

Differences in response to different FA were probably not due to differential binding of FA to albumin, because no detectable unbound FA was found in the medium with any of the FAs used. The different FAs did not cause ketoacidosis or cytotoxicity in the Hep G2 cells at the concentrations employed, as determined by beta-hydroxybutyrate and, LDH determinations, which were not significantly different for any fatty acid and were not different from control incubations (results not shown).

3.2. Saturated fatty acids

Lauric acid (C12:0) caused a small, non-significant increase in apo B secretion from Hep G2 cells (36%) (Fig. 4), but myristic acid (C14:0) caused apo B secretion to be significantly raised when compared to controls (46%, P < 0.005), as did palmitic acid (C16:0) (154%, P < 0.025). Stearic acid (C18:0), however, did not change apo B secretion compared to control values. Arachidic acid (C20:0), was found to increase apo B secretion by Hep G2 cells more than any of the other SFAs (186%, P < 0.005).

When cells were incubated with SFA, only those incubated with stearic acid showed significantly increased intracellular concentrations of CE (345%, P < 0.0005) (Fig. 5).
Fig. 4. Apolipoprotein B accumulation in the medium from Hep G2 cells after incubation with different fatty acids. Experimental details are described under Section 2. Values are Mean ± SE of 15 experiments. Significantly different from 1% BSA, * \( P < 0.05 \), ** \( P < 0.005 \), *** \( P < 0.0005 \).

Fig. 5. Intracellular cholesteryl ester (CE) accumulation after incubation with different fatty acids. Experimental details are described under Section 2. Values are Mean ± SE of 15 experiments. Significantly different from 1% BSA, * \( P < 0.05 \), ** \( P < 0.005 \), *** \( P < 0.0005 \).

SFA chain length influenced the intracellular TG accumulation (Fig. 6). Thus, intracellular TG concentration in the presence of lauric acid and myristic acid was not significantly increased, whereas it was by palmitic acid \( (P < 0.01) \), arachidic acid \( (P < 0.005) \) (both by 130%) and stearic acid \( (P < 0.005) \) by 100%.

### 3.3. Monounsaturated fatty acids

Oleic acid (C18:1) significantly increased apo B production above the control value \( (239\%, P < 0.005) \) (Fig. 4), as did palmitoleic acid (C16:1) \( (125\%, P < 0.005) \). Trans-vaccenic acid (C18:1) is a trans-isomer and it
did not have any significant effect on apo B production.

All the MUFA studied increased the CE and TG content of Hep G2 cells (Figs. 5 and 6) to varying degrees.

3.4. Polyunsaturated fatty acids

Linoleic acid (C18:2) significantly increased apo B production by 164%, \( P < 0.005 \) as did arachidonic (C20:4) and eicosapentaenoic (C20:5) acids, both by 171% \( (P < 0.0005) \) (Fig. 4). Linolenic acid (C18:3), however, did not significantly affect apo B production. All of the PUFA investigated caused significant increases in the CE content of the cells (Fig. 5). The increases ranged from 627% \( (P < 0.0005) \) for linolenic acid and 418% for linoleic acid \( (P < 0.0005) \) to 127% for both eicosapentaenoic and arachidonic acid \( (P < 0.025) \). All the PUFA also increased intracellular TG (Fig. 6). Both arachidonic acid and eicosapentaenoic increased intracellular TG by 140% \( (P < 0.005) \), while linolenic acid caused a 100% increase \( (P < 0.01) \). The greatest increase was observed with linoleic acid (240%, \( P < 0.0005) \).

3.5. Relationship between intracellular triacylglycerol and cholesteryl ester content and the rate of apo B secretion

There was a significant \( (P < 0.05) \) correlation \( (r = 0.6) \) between Hep G2 cell TG content after incubations with the various FAs and the rate of apo B secretion into the medium (Fig. 7a). The correlation coefficient \( (r = 0.32) \) between Hep G2 cell cholesteryl ester content and apo B secretion did not, however, achieve statistical significance (Fig. 7b).

4. Discussion

Apo B accumulation in the medium of Hep G2 cells was chosen as a measure of VLDL secretion because of our finding, reported earlier [14] and confirmed in these experiments, that TG rapidly undergoes lipolysis after its secretion into the culture medium, presumably by hepatic lipase, which is more actively expressed by Hep G2 cells than, for example, by adult rat hepatocytes in tissue culture [14,19].

Our results show that when VLDL secretion is assessed in terms of TG accumulation in the medium, no apparent increase is observed with increasing FA concentration, whereas measurement of apo B accumulation clearly shows secretion of apo B-containing lipoproteins to be stimulated. Previous investigations are divided about whether oleic acid and eicosapentaenoic acid stimulate VLDL secretion by Hep G2 cells, but any positive effects are not marked [8,20,21]. To explain the lack of apparent stimulation of TG secretion by FA in Hep G2 cells, it has been suggested that VLDL secretion by hepatocytes is defective and that the dense, TG deficient, apo B-containing lipoproteins present in the culture medium are secreted in that form by the Hep G2 cells [22,23]. Our results suggest that an alternative explanation for these observations is that there is rapid lipolysis of TG in VLDL after its secretion. This conclusion is also supported by our earlier
study in which there was rapid reuptake of radioactively labelled fatty acids in TG secreted by Hep G2 cells into the culture medium [14].

It is well accepted that dietary SFA with the exception of stearate increase plasma TC and LDL-C concentrations, whereas unsaturated fatty acids such as oleate and linoleate lower serum TC and LDL-C and the more unsaturated eicosapentanoate and docosahexaenoate found in fish-oils lower TG and VLDL-C [5,20,24]. This forms the basis of current dietary recommendations to decrease serum cholesterol and TG [1]. In the experiments reported here, SFAs with the exception of stearate increased apo B secretion by Hep G2 cells. The effects of SFA on the secretion of apo B-containing lipoproteins in our human hepatocyte model thus reflect the dietary effects of SFAs on serum concentrations of these lipoproteins, including the neutral effect of stearate [20]. However, the MUFAs and PUFAs investigated with the exception of linolenic acid and vaccenic acid, which is a trans-FA, also increased the secretion of apo B-containing lipoproteins.

Our finding that there was a strong positive relationship between intracellular TG accumulation and apo B secretion would suggest that the ability of a FA to stimulate TG synthesis is the major determinant of its ability to stimulate apo B secretion. We found only a weaker non-significant relationship between cholesteryl ester (CE) synthesis and apo B secretion, although
some investigators have suggested that CE is the major lipid species stimulating VLDL secretion by Hep G2 cells [25]. It is possible that in the presence of abundant FA for TG synthesis, CE becomes less important. Furthermore it is well-known that in the human very little CE is secreted in hepatic VLDL, most of the cholesterol secreted by the liver being in the form of free cholesterol, esterification of which occurs in the circulation [18]. Thus the situation in human liver, unlike for example that in the rat in which most cholesterol is esterified before secretion, may mean that hepatic TG synthesis has a greater influence on apo B secretion. Our study thus supports the conclusion of Benoist and Grand-Perret [26] using inhibitors of lipid biosynthesis in Hep G2 cells.

Conclusions based on the Hep G2 cell model of lipoprotein secretion must necessarily be limited, because it is an immortalised cell line derived from neoplastic tissue. However, the present observations of the differential effects of different fatty acids on apo B secretion by Hep G2 cells are interesting, firstly because they indicate that TG synthesis is the dominant influence on apo B secretion in human hepatocytes and secondly because the primary effect of FAs on apo B secretion does not reflect their dietary effects on serum VLDL and LDL levels in human experiments. The implications of this are that the nutritional effects of different fatty acid components in the diet on serum apo B-containing lipoproteins are likely to include indirect effects on hepatic lipoprotein secretion or post-secretory effects. The mechanism by which polyunsaturated fatty acids decrease hepatic TG secretion [27] may, for example, be due to decreased circulating NEFA levels [28] which are a major determinant of hepatic TG synthesis [29]. Recent reviews have also emphasised the importance of the decreased receptor-mediated catabolism of LDL during saturated fatty acid feeding [30] and the increase in cholesteryl ester transfer from HDL to apo B-containing lipoproteins [31]. The high rates of apo B secretion induced by highly polyunsaturated fatty acids in our experiments might also explain why, in human feeding experiments, eicosapentaenoic acid and docosahexaenoic acid do not lower cholesterol, despite their potentially favourable effect on LDL receptor-mediated clearance. On the other hand less highly unsaturated fatty acids, such as linoleate and oleate, do lower serum LDL cholesterol levels, perhaps because they stimulate VLDL and LDL production to a lesser extent, thus allowing their favourable effect on LDL receptor mediated clearance to come to the fore.

Hep G2 cells cultured in flasks are not a suitable model for the investigation of the effects of different fatty acids on TG secretion, because of high rates of lipolysis when TG-containing lipoproteins remain in contact with the cells. However, our preliminary experiments and those of others [16] suggest that perfused Hep G2 may provide a suitable method for studying TG metabolism and other aspects of lipoprotein metabolism in future studies.

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