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Regulation of hepatic synthesis and secretion of cholesterol and glycerolipids in animals maintained in different nutritional states

Julia M. DUERDEN, Brian MARSH, Faith J. BURNHAM and Geoffrey F. GIBBONS*
Metabolic Research Laboratory, Nuffield Department of Clinical Medicine, Radcliffe Infirmary, Woodstock Road, Oxford OX2 6HE, U.K.

The distribution of newly synthesized and exogenous fatty acids and of newly synthesized cholesterol between cellular and very-low-density lipoprotein (VLDL) lipids was studied in hepatocytes derived from animals fed on a normal diet or on diets supplemented with polyunsaturated fat or sucrose. Phospholipid synthesis from either exogenous or endogenous (biosynthetic) fatty acids was unaffected by nutritional state. Cholesterol synthesis was decreased in the fat-fed animals, but sucrose feeding had no significant effect. In all nutritional states, newly synthesized rather than exogenous fatty acids were better substrates for phospholipid synthesis. In all groups, compared with newly synthesized triacylglycerol, smaller proportions of newly synthesized phospholipid and cholesterol were secreted as VLDLs. This was confirmed in intact animals by using Triton WR-1339. Newly synthesized phospholipid formed a greater proportion of the VLDL glycerolipid in the fat-fed than in the normal or sucrose-fed animals. In all groups, phospholipids labelled from endogenous fatty acids were secreted in preference to those labelled from exogenous fatty acids.

INTRODUCTION

The liver synthesizes triacylglycerol primarily for utilization by extrahepatic tissues. This is achieved by the assembly and secretion of very-low-density lipoprotein (VLDL) [1]. Although cholesterol and phospholipids are essential for this process [2,3], these lipids are also required by the liver for other purposes, notably membrane biogenesis and as components of the bile. Changes in nutritional state give rise to changes in the rate of hepatic VLDL triacylglycerol secretion, which appears to reflect changes in the extrahepatic demand for hepatically synthesized triacylglycerol. For instance, diets supplemented with fat or sucrose respectively decrease [4,5] or increase [6,7] the rate of VLDL triacylglycerol output. These types of changes are accompanied by corresponding variations in the rates at which triacylglycerol is synthesized *de novo* [6,8] and the incorporation of this newly synthesized glycerolipid into VLDL [1]. Because of the multifunctional roles of cholesterol and phospholipid, changes in their rates of synthesis and utilization for VLDL assembly in response to nutritional changes may not be so clear-cut as that for triacylglycerol. One of the aims of the present work is therefore to determine how changes in nutritional state affect the secretion of newly synthesized phospholipid and cholesterol, reflecting the requirements for VLDL synthesis on the one hand, and their retention by the liver, reflecting their requirements for other purposes, on the other. Although there is a substantial amount of information relating to the differential utilization of various exogenously added fatty acids for hepatic phospholipid synthesis [9–11], little information is currently available as to whether the liver discriminates between exogenous (extracellular) and newly synthesized (*de novo* biosynthetic) fatty acids in this respect. There has been only one systematic study of this aspect [12], but the total mass of newly synthesized fatty acids that was esterified was not measured. The question as to whether a particular fatty acid which becomes available to the liver in a newly synthesized form is handled differently from the same fatty acid presented extracellularly has not been addressed in the present work. The point at issue is whether the liver discriminates between fatty acids synthesized *de novo* and extracellular fatty acid, present *in vivo* as plasma NEFA as a source of substrate for phospholipid synthesis. We have used the hepatocyte model to investigate this aspect, using oleate as a representative extracellular fatty acid. This approach has been used previously, utilizing the isolated perfused liver model [13,14]. The present study has been designed to compare the response of glycerolipid synthesis to changes in the availability of fatty acids, synthesized *de novo* and exogenous, in hepatocytes isolated from rats maintained in various nutritional states. The total mass of newly synthesized fatty acids incorporated was determined by using ^3H_2O, and their availability *in vitro* was varied by changing the concentration of lactate plus pyruvate [15–17]. This resulted in a several-fold change in the rate of carbon flux into fatty acids.

MATERIALS AND METHODS

Maintenance of animals and preparation of hepatocytes

Male Wistar rats were housed in a windowless room which was artificially lit on a 12 h-dark/12 h-light alternating schedule (lights on 16:00 h; lights off 04:00 h). Animals were accustomed to this schedule for at least 10 days. At the end of this period rats were assigned to one of three groups. One group was fed *ad libitum* with a chow diet obtained from a commercial source (Diet 41B; Dixon and Sons, Ware, Herts., U.K.) [18]. A second group was fed on a diet in which the powdered chow diet was supplemented with 20% (w/w) corn oil, followed by reconstitution as pellets [19]. The third group was fed on the chow diet and was simultaneously allowed unrestricted access to a solution of sucrose in the drinking water (25%, w/v) [6,20]. These diets were given *ad libitum* for 7 days before hepatocyte preparation. The average food consumption of rats on the chow diet and the fat-supplemented diet was 25 g/day. The sucrose-supplemented rats consumed 17 g/day and, in addition, drank sucrose solution equivalent to an average daily sucrose intake of 8.1 g. Hepatocytes were prepared at 10:00 h (i.e. the mid-point of the dark phase of the cycle) as described previously [21,22].

Abbreviations used: VLDL, very-low-density lipoprotein; NEFA, non-esterified fatty acid.
* To whom correspondence should be addressed.

Incubation of hepatocytes

Hepatocytes were incubated in Krebs–Henseleit [23] bicarbonate buffer supplemented with 3.5% (w/v) BSA, glucose (11.1 mM) and amino acids [24] in a total volume of 3.0 ml. In those incubations in which triacylglycerol was formed from newly synthesized fatty acids, the medium was supplemented with mixtures of [^{14}C]lactate and unlabelled pyruvate (10:1 ratio) in which the initial lactate concentrations were 0 (tracer only), 2, 5 and 10 mM. The specific radioactivities of the lactate precursors were 169, 0.167, 0.067 and 0.033 μCi/μmol respectively. Total rates of fatty acid synthesis were determined in separate incubations containing ^3H_2O and [^{14}C]lactate. This information was then used to calculate the contribution of fatty acids, synthesized from all sources of carbon, to cellular and VLDL glycerolipid fatty acids (see below). Where exogenous fatty acids were the triacylglycerol precursor, the medium was supplemented with [^{3}H]oleate (1.33 μCi/μmol) at concentrations of 0.25, 0.50 and 0.75 μM. Incubations were carried out for 3 h under an atmosphere of O_2/CO_2 (19:1). We have shown previously that rates of secretion of VLDL and rates of fatty acid synthesis did not decline during 3 h under these conditions [17,21]. The initial viabilities of cells from each group of rats were greater than 90% and did not decrease significantly during the incubation period.

Isolation of cellular and VLDL lipids

The cell pellet was removed from the incubation suspension by sedimentation at 80 *g* for 1.5 min, and the VLDL fraction ($d <$ 1.006) was isolated from the cell supernatant as described previously [5]. The cellular and VLDL lipids were extracted by the method of Folch *et al.* [25], and a portion of each extract was used for measurement of the total mass of triacylglycerol [26]. Labelled triacylglycerol, cholesterol and phospholipids were purified from the remaining lipid extract by t.l.c. on plates of silica gel G by using hexane/diethyl ether/acetic acid (70:20:1, by vol.) [27]. Samples of each lipid fraction were removed for measurement of radioactivity, and the remainder of the triacylglycerol fraction was hydrolysed. The triacylglycerol fatty acids were obtained by solvent extraction and their radioactivity was determined. Manipulative losses were accounted for by addition of glycerol [^{3}H]trioleate as internal standard to the VLDL and cells labelled from [^{14}C]lactate, and of glycerol [^{14}C]trioleate to those labelled from [^{3}H]oleate.

Calculations of the contribution of newly synthesized fatty acids to cellular and VLDL glycerolipids and measurement of rates of cholesterol synthesis

The use of [^{14}C]lactate alone gives no information as to the total quantity of newly synthesized fatty acids utilized for glycerolipid synthesis. To overcome this problem, separate and parallel incubations were carried out at the various concentrations of lactate. In these cases, however, ^3H_2O (26.4 d.p.m./nmol) was also present in addition to [^{14}C]lactate. After 3 h of incubation, the cells were isolated by a brief centrifugation, and the labelled non-saponifiable and saponifiable fractions were isolated as described previously [28]. Labelled cholesterol was isolated from the non-saponifiable lipid by t.l.c. [28] and its ^{3}H and ^{14}C radioactivities were determined. The mass of newly synthesized cholesterol (nmol) was calculated from the incorporation of ^3H_2O as described previously [22]. The saponifiable fraction of the cell contained the newly synthesized fatty acids, the mass of which was calculated from the incorporation of ^3H_2O by using the relationship determined by Jungas [29]. This provided information about the flux of carbon from all sources into fatty acids and reflects the total mass of newly synthesized fatty acids (nmol). Simultaneous measurement of ^{14}C incorporation from [^{14}C]lactate allowed calculation of the ^{14}C specific radioactivity of the total newly synthesized fatty acids (^{14}C d.p.m./nmol). By using these values, the incorporation of ^{14}C from [^{14}C]lactate into VLDL and cellular glycerolipids, measured in parallel incubations without ^3H_2O, could be applied to calculate the incorporation of total newly synthesized fatty acids. This strategy was adopted to avoid the use of ^3H_2O in incubations from which VLDL was isolated. This eliminated the risk of ^{3}H contamination during centrifugation at high speeds followed by tube-slicing.

The contribution of the glycerol moiety to the labelled triacylglycerol was calculated by the difference before and after hydrolysis. This contribution was deducted from the labelled phospholipid isolated after t.l.c. after allowing for the difference in the molar content of fatty acid between phospholipid and triacylglycerol. The balance of radioactivity was then associated with the phospholipid fatty acids. This calculation, of course, assumes that the pool of glycerol utilized for phospholipid and triacylglycerol synthesis is identical, an assumption which is implicit in the widespread use of [^{3}H]glycerol to measure relative rates of triacylglycerol and phospholipid synthesis.

Determination of the contribution of newly synthesized hepatic lipids to VLDL *in vivo*

Animals were removed from their cages at the mid-point of the dark phase of the diurnal cycle (10:00 h) and injected simultaneously with ^3H_2O (intraperitoneally) and a solution of Triton WR-1339 (intravenously) [18]. Then 3 h later the animals were anaesthetized, a blood sample (2.0 ml) was removed from the descending vena cava and, at the same time, the liver was removed. VLDL was isolated from the blood plasma and the VLDL lipids were extracted and purified by t.l.c. [18]. The cholesterol, triacylglycerol and phospholipid fractions thus isolated were assayed for ^{3}H radioactivity by scintillation counting. The total lipid fraction was isolated from a sample of liver [18], and the labelled cholesterol, phospholipid and triacylglycerol fractions were obtained in the same way as described above for VLDL. The sum of the radioactivity in the hepatic plus VLDL triacylglycerol was considered to represent the total triacylglycerol newly synthesized during a 3 h period *in vivo*. The fraction of this total which appeared in the VLDL then represented the proportion of this lipid that had been secreted during the 3 h period. Similar calculations were also carried out to determine the proportions of newly synthesized cholesterol and phospholipid which were secreted as VLDL.

Materials

All radiochemicals were obtained from Amersham International (Little Chalfont, Bucks., U.K.). [^{3}H]Oleate was bound to fatty-acid-free BSA (Sigma Chemical Co., Poole, Dorset, U.K.) [30] before addition to the hepatocyte suspensions. Corn oil was purchased locally, and its composition was as described previously [19].

RESULTS

Cellular glycerolipid synthesis from endogenous and exogenous fatty acids

Hepatocytes were prepared from normal (chow-fed) rats and from rats fed on diets supplemented with sucrose or with polyunsaturated fat. Cells from each of these groups were incubated with increasing concentrations of exogenous [^{3}H]oleate bound to albumin. To increase the supply of biosynthetic fatty acids, cells from the same animals were incubated under identical conditions except that various concentrations of [^{14}C]lactate (plus unlabelled pyruvate) were present in the medium. This has

Table 1. Effect of endogenous (biosynthetic) and exogenous fatty acids on cellular phospholipid synthesis

Hepatocytes were incubated for 3 h in the presence of [^{14}C]lactate or [^{3}H]oleate, and the labelled cellular triacylglycerol and phospholipid fractions were isolated. Values represent the incorporation of newly synthesized fatty acids (lactate precursor) or of exogenous fatty acids (oleate precursor). Unless otherwise indicated, each value represents the mean ± S.E.M. of three independent hepatocyte preparations. Values marked * are significantly different ($P < 0.05$) from those observed in the presence of 2 mM-lactate. Values marked † are significantly different ($P < 0.05$) from those in the presence of 10 mM-lactate.

Nutritional state	Lipid precursor	Phospholipid synthesis (nmol of fatty acid incorporated/mg of protein)	Phospholipid fatty acids (% of total glycerolipid fatty acids)
Chow-fed	2 mM-Lactate	10.48 ± 1.04 (3)	61.6 ± 6.2
	10 mM-Lactate	14.19 ± 1.82 (5)	59.5 ± 3.8
	0.25 mM-Oleate	2.83 ± 1.10 (5)*†	34.7 ± 7.0*†
	0.50 mM-Oleate	6.27 ± 2.63†	41.0 ± 12.6
	0.75 mM-Oleate	9.51 ± 2.79 (4)	40.9 ± 8.0†
Polyunsaturated-fat-fed	2 mM-Lactate	10.30 ± 0.32 (2)	71.6 ± 4.9
	10 mM-Lactate	9.50 ± 2.17	65.7 ± 3.8
	0.25 mM-Oleate	2.97 ± 1.04 (4)*†	47.3 ± 4.7*†
	0.50 mM-Oleate	3.95 ± 1.43 (2)	40.7 ± 6.3
	0.75 mM-Oleate	8.53 ± 2.79 (4)	45.7 ± 7.7
Sucrose-fed	2 mM-Lactate	6.63 ± 1.21	58.7 ± 4.6
	10 mM-Lactate	8.27 ± 4.43	36.8 ± 14.7
	0.25 mM-Oleate	2.59 ± 0.60*	34.9 ± 8.3*
	0.50 mM-Oleate	5.16 ± 1.80	32.4 ± 10.9
	0.75 mM-Oleate	9.71 ± 4.45	34.3 ± 12.4

Table 2. Secretion of newly synthesized glycerolipids with VLDL

Hepatocytes were incubated for 3 h as described in the legend to Table 1. After 3 h, the secreted VLDL was isolated and the radioactivity of the glycerolipid fatty acids determined. Unless otherwise indicated, each value represents the mean ± S.E.M. of three independent hepatocyte preparations: *, **, ***, significant difference from 2 mM-lactate ($P < 0.05$, $P < 0.01$, $P < 0.001$ respectively); †, ††, †††, significant difference from 10 mM-lactate ($P < 0.05$, $P < 0.01$, $P < 0.001$ respectively).

Nutritional state	Glycerolipid precursor	Secretion of newly synthesized phospholipid (nmol of fatty acid per mg of protein)	Phospholipid fatty acids (% of total glycerolipid fatty acids secreted)
Chow-fed	2 mM-Lactate	0.36 ± 0.05	16.5 ± 4.6
	10 mM-Lactate	1.16 ± 0.64 (4)	24.5 ± 9.5
	0.25 mM-Oleate	0.028 ± 0.007 (5)***	2.1 ± 0.8**†
	0.50 mM-Oleate	0.16 ± 0.06	8.4 ± 4.5
	0.75 mM-Oleate	0.058 ± 0.023 (5)***	1.0 ± 0.4**†
Polyunsaturated-fat-fed	2 mM-Lactate	0.91 ± 0.43 (2)	51.3 ± 9.2
	10 mM-Lactate	0.78 ± 0.37 (4)	43.0 ± 8.1
	0.25 mM-Oleate	0.018 ± 0.009 (4)*†	3.9 ± 1.3***††
	0.50 mM-Oleate	0.05 ± 0.01 (2)	5.0 ± 1.0*†
	0.75 mM-Oleate	0.07 ± 0.02 (2)	3.8 ± 0*†
Sucrose-fed	2 mM-Lactate	0.46 ± 0.22	17.9 ± 12.0
	10 mM-Lactate	0.71 ± 0.69	3.7 ± 2.6
	0.25 mM-Oleate	0.01 ± 0.01	0.13 ± 0.13
	0.50 mM-Oleate	0.05 ± 0.03	1.67 ± 1.61
	0.75 mM-Oleate	0.03 ± 0.03	0.36 ± 0.36

been previously shown to increase total carbon flux into fatty acids from hepatocytes from normal and starved animals [15–19]. In the present work, parallel incubations carried out in the presence of [^{14}C]lactate and $^{3}H_2O$ showed that, compared with basal levels after a 3 h incubation, 10 mM-lactate increased the incorporation of $^{3}H_2O$ into fatty acids by 2.94-fold, 3.04-fold and 2.94-fold in the cells from the control, fat-fed and sucrose-fed animals respectively. Table 1 shows that, in general, apart from the small decrease with 10 mM-lactate in the fat-fed animals, increasing the availability of fatty acids from either the exogenous or the endogenous source led to an increase in the mass of phospholipids synthesized. However, increasing the availability of endogenous fatty acids did not lead to any significant change in the relative amounts which were esterified to phospholipid compared with triacylglycerol. In other words, there was no change in the relative contribution of phospholipid to total glycerolipid synthesis. The same was true when the concentration of exogenous fatty acid was increased. However, when endogenous fatty acids were the substrate, phospholipids were the major esterified product, whereas this was not the case when

exogenous oleate was used as the glycerolipid precursor. In each nutritional group, especially at low concentrations of oleate, the mass of exogenous fatty acid incorporated into phospholipid was significantly less than that of newly synthesized fatty acid (Table 1). Feeding the polyunsaturated-fat- or sucrose-supplemented diets had little, if any, effect on the rate of phospholipid synthesis from either precursor compared with that observed in animals fed on the chow diet.

Utilization of newly synthesized phospholipid and non-esterified cholesterol for VLDL secretion

Despite the predominance of newly synthesized phospholipid within the cellular glycerolipid fraction when endogenous fatty acids were utilized as substrate, phospholipids contributed a relatively minor proportion of the newly synthesized glycerolipids which were secreted as VLDL (Table 2). This was the case regardless of nutritional state. Nevertheless, in the polyunsaturated-fat-fed animals, phospholipid contributed a greater proportion of the newly synthesized glycerolipid secreted with the VLDL compared with that in the chow-fed or sucrose-fed groups. In the sucrose-fed animals, at 10 mM-lactate compared with 2 mM-lactate, the proportion of labelled glycerolipid secreted as phospholipid declined. This was due to the large increase in the proportion of VLDL triacylglycerol that was secreted under these conditions. When phospholipids were synthesized from exogenous [^{3}H]oleate, the appearance of label in the VLDL phospholipid was very low indeed, despite their relatively large contribution to the cellular glycerolipid under these conditions. Thus the utilization, for VLDL secretion, of those phospholipids synthesized from exogenous oleate was much less than that of phospholipid synthesized from endogenous fatty acids (Table 2). The low, and sometimes barely detectable, level of incorporation of exogenous fatty acid into VLDL phospholipid led to large variations, and this is reflected by the large S.E.M. values (Table 2). Thus the apparent increase in exogenous fatty acid incorporation at 0.5 mM-oleate is minuscule and of no significance.

The overall pattern of glycerolipid synthesis and secretion in hepatocytes was such that a much larger proportion of the total triacylglycerol synthesized was secreted as VLDL compared with the total newly synthesized phospholipid, the overwhelming amount of which was retained within the cell (Table 3). Parallel measurements of the distribution of newly synthesized cholesterol between cells and VLDL revealed a pattern which was intermediate between that for triacylglycerol and phospholipid (Table 4). Feeding the sucrose-rich diet had no significant effect on the rate of cholesterol synthesis compared with the chow-fed group. Dietary supplementation with polyunsaturated fat decreased the rate of cholesterol synthesis, as reported previously [19]. However, within this group, in contrast with its lack of effect in hepatocytes from the chow-fed and sucrose-fed groups, lactate added to the medium of the cells increased carbon flux into cholesterol [143.1 ± 12.6% at 10 mM-lactate compared with zero lactate (100%); $n = 8$, $P < 0.01$]. Dietary manipulation did not appear to have any significant effect on the proportion of newly synthesized cholesterol which was secreted as VLDL. Nor, in this respect, was there any significant effect of lactate, added *in vitro*, in any of the different dietary groups (Table 4).

The distribution of newly synthesized cholesterol and phospholipid between the liver and the secreted VLDL was also investigated *in vivo*, in chow-fed animals, by treating rats simultaneously with Triton WR-1339 and ^3H_2O. Triton blocks the peripheral catabolism of VLDL [31], and thus its lipid constituents, newly synthesized by the liver from ^3H_2O administered intraperitoneally [18,32], accumulate in the plasma. Measurement of hepatic and plasma VLDL cholesterol, phospholipid and triacylglycerol then provides information about the secretion and hepatic retention of these lipids. The results of this experiment are shown in Table 5. Of the total newly synthesized cholesterol and phospholipid,

Table 3. Proportion of newly synthesized glycerolipids which are secreted as VLDL: effect of nutritional state

Hepatocytes were incubated for 3 h in the presence of various concentrations of [^{3}H]oleate. After this time the VLDL and cellular triacylglycerol and phospholipids were isolated. The sum of the radioactivity in the cellular and VLDL triacylglycerol fatty acids represented the total amount of triacylglycerol synthesized during this period. The percentage of this total which was secreted as VLDL was then determined. A similar calculation was used to determine the percentage of the total synthesized phospholipids which was secreted as VLDL.

Nutritional state	Oleate concn. (mM)	VLDL glycerolipids secreted (% of total synthesized)	
		Phospholipid	Triacylglycerol
Chow-fed	0.25	1.39±0.39 (5)	23.7±4.0 (5)
	0.50	2.77±1.05 (3)	20.7±1.8 (3)
	0.75	0.98±0.55 (4)	31.8±2.1 (4)
Polyunsaturated-fat-fed	0.25	0.78±0.37 (4)	22.3±3.6 (5)
	0.50	1.54±0.56 (2)	17.5±3.3 (2)
	0.75	0.77±0.33 (2)	20.0±4.3 (3)
Sucrose-fed	0.25	0.7±0.7 (3)	34.1±5.3 (3)
	0.50	2.4±2.0 (3)	32.9±6.6 (3)
	0.75	1.3±1.3 (3)	25.6±5.6 (3)

Table 4. Proportion of newly synthesized cholesterol which is secreted as VLDL

Hepatocytes were incubated for 3 h with various concentrations of [^{14}C]lactate (plus pyruvate) in the presence of ^3H_2O., The total mass of the newly synthesized cellular cholesterol was calculated from the incorporation of ^{3}H (1 nmol of newly synthesized cholesterol ≡ 7.61 nmol of ^3H_2O). Incorporation of [^{14}C]lactate into cholesterol was also measured, and the ^{14}C specific radioactivity of the newly synthesized cholesterol was determined. Parallel incubations were carried out in the absence of ^3H_2O. In this case the VLDL cholesterol was isolated and its ^{14}C radioactivity determined. This value, together with the ^{14}C specific radioactivity of the newly synthesized cholesterol, was used to calculate the mass of newly synthesized cholesterol which was secreted as VLDL. The value marked with an asterisk is significantly different ($P < 0.05$) from the control value in the absence of added lactate.

Nutritional state	Lactate concn. (mM)	Cellular cholesterol synthesis (nmol/mg of protein)	VLDL cholesterol secreted (% of total synthesized)
Chow-fed	0	2.7±0.6 (9)	8.9±2.2 (5)
	2.0	2.8±0.8 (7)	9.9±2.4 (3)
	5.0	3.1±0.9 (7)	8.7±3.3 (3)
	10.0	3.1±0.6 (8)	11.1±1.8 (4)
Polyunsaturated-fat-fed	0	1.4±0.3 (8)*	12.9±4.4 (4)
	2.0	1.9±0.5 (6)	6.6±1.4 (2)
	5.0	2.2±0.4 (6)	8.7±0.5 (2)
	10.0	1.9±0.4 (8)	9.0±1.7 (4)
Sucrose-fed	0	3.5±0.4 (4)	7.6±1.5 (3)
	2.0	3.7±0.5 (3)	9.5±2.8 (3)
	5.0	3.6±0.4 (4)	13.0±0.7 (3)
	10.0	3.7±0.5 (4)	18.0±5.3 (3)

Table 5. Secretion and retention of newly synthesized hepatic lipids *in vivo*

Rats were injected intraperitoneally with ^3H_2O simultaneously with a tail-vein injection of Triton WR-1339; 3 h later, samples of blood and liver were removed. After isolation of the plasma VLDL, labelled cholesterol, phospholipid and triacylglycerol were isolated by t.l.c. For cholesterol and triacylglycerol, the VLDL and liver lipids were obtained from the same animals. For phospholipid, different animals were used for the VLDL and liver assays. The values represent the averages ± S.E.M. for at least three rats.

Biosynthetic lipid	Amount associated with liver (μmol of ^3H_2O/g)	Amount associated with VLDL (μmol of ^3H_2O secreted/g of liver)	Amount associated with VLDL (% of total)
Cholesterol	8.8 ± 1.6	1.3 ± 0.1	13.9 ± 3.1
Phospholipid	11.2 ± 2.0	1.4 ± 0.2	11.3
Triacylglycerol	14.7 ± 1.3	23.2 ± 3.7	61.8 ± 3.7

only about 10–15% was secreted with the VLDL, by far the greatest proportion being retained by the liver. However, more than 50% of the newly synthesized triacylglycerol was secreted as VLDL during the 3 h study period. Although the proportion of each lipid secreted was somewhat higher than that observed *in vitro* (Tables 3 and 4), the overall relative secretion pattern was broadly similar.

DISCUSSION

The present work shows that, in general, rates of hepatic phospholipid and cholesterol synthesis are relatively resistant to changes in the content of polyunsaturated fat or sucrose in the diet. This contrasts with the effects of these dietary manipulations on fatty acid [19] and triacylglycerol synthesis [6–8]. The only notable exception was the inhibitory effect of polyunsaturated fat on cholesterol synthesis, a decrease which was partially rectified by providing lactate *in vitro*. Whereas hepatic triacylglycerol is ultimately used exclusively for export as VLDL, phospholipid and cholesterol serve, in addition, other purposes as membrane constituents and as biliary lipids [33]. The requirements of phospholipid and cholesterol for these purposes are, presumably, relatively resistant to changes resulting from dietary manipulation. The relatively low incorporation of newly synthesized phospholipid and cholesterol into VLDL also reflects the important role of these lipids in processes other than VLDL synthesis.

The large amounts of newly synthesized triacylglycerol which are associated with secreted VLDL (Tables 3 and 5) suggest a rapid transfer of this material into the lumen of the secretory apparatus as part of the incipient VLDL [34]. The present work shows for the first time that, both *in vivo* and *in vitro*, compared with newly synthesized triacylglycerol, much smaller proportions of the total newly synthesized cholesterol and phospholipid are associated with the secreted VLDL (Tables 3, 4 and 5). It therefore appears that whatever the topographical site(s) of cholesterol and phospholipid synthesis, most of these lipids remain associated with intracellular membranes rather than undergoing transfer into the lumen of the secretory apparatus for participation in VLDL assembly. As regards cholesterol, this is supported by evidence that the newly synthesized sterol equilibrates with the bulk of the cellular cholesterol pool before incorporation into VLDL [35]. In contrast, a large proportion of the newly synthesized triacylglycerol becomes associated with VLDL without prior equilibration with cellular material [18, 36–38].

There is considerable evidence that phospholipids are both synthesized and incorporated into the developing VLDL within the Golgi apparatus [33,37,39,40]. If this is the case, the present work provides evidence that newly synthesized (endogenous) rather than extracellular fatty acids are better substrates for phospholipid synthesis at this site (Table 1). This conclusion is broadly in agreement with that of Groener & Van Golde [12], who also showed that, at high concentrations of extracellular palmitate, this substrate was incorporated relatively less efficiently into phospholipids, compared with triacylglycerols, in contrast with fatty acids newly synthesized from labelled acetate and lactate. Azain *et al.* [14], using extracellular oleate as the exogenous source of fatty acid, also showed that biosynthetic fatty acids were preferentially incorporated into cellular phospholipids. The present work extends these previous reports and highlights the effects of unsaturated-fat feeding on the utilization of newly synthesized and exogenous fatty acids for cellular- and VLDL-phospholipid synthesis. There are several possible explanations for the present observations: (*a*) newly synthesized fatty acids have easier access to the phospholipid substrate pool than do exogenous fatty acids; (*b*) newly synthesized fatty acids are more effective in translocating CTP–phosphocholine cytidylyltransferase (EC 2.7.7.15), an important regulatory enzyme, from the cell cytoplasm, where it is inactive, to the particulate fraction of the cell, where it becomes catalytically active [41,42]; (*c*) specificity of the enzymes involved in phospholipid synthesis may result in structural discrimination between oleate and newly synthesized fatty acids, the largest proportion of which are saturated. This explanation, however, seems unlikely in view of the observations, similar to the present results, reported by Groener & Van Golde [12], who used palmitate as the extracellular source of fatty acid.

Phospholipids synthesized from '*de novo* biosynthetic' fatty acids contributed a small but significant proportion of the total newly synthesized VLDL glycerolipid, particularly in the fat-fed animals (Table 2). However, the almost complete absence of secreted phospholipid synthesized from exogenous oleate implies some kind of specificity in the selection of phospholipids destined for secretion, which is dependent on their synthetic origin. Vance and colleagues [42] have shown that phosphatidylcholine constitutes 75% of the total VLDL phospholipid in rats and, further, that the synthesis of this class of phospholipid is an absolute requirement for hepatic VLDL secretion [2]. A possible explanation for the present findings is that phosphatidylcholine is selectively labelled from newly synthesized fatty acids and that the hepatocyte discriminates between this and other classes of phospholipid, in the assembly of VLDL.

In general, little is known of the major route(s) by which exogenous and newly synthesized fatty acids are channelled into cellular and VLDL glycerolipids. As regards triacylglycerol, *in vivo* over 50% of the newly synthesized fatty acids incorporated into triacylglycerol are secreted as VLDL (Table 5). However, this represents only a minor contribution to the total mass of the VLDL triacylglycerol secreted [18]. In this respect, the importance of cytosolic triacylglycerol in providing fatty acids for the synthesis of VLDL triacylglycerol has been emphasized [14,43]. The extent to which cellular phospholipids contribute to this process [14] is an interesting but, as yet, unanswered question.

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