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Effect of nutritional state on the utilization of fatty acids for hepatic triacylglycerol synthesis and secretion as very-low-density lipoprotein

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The mass of very-low-density-lipoproteins (VLDL) triacylglycerol secreted from isolated hepatocytes was dependent on the nutritional state of the donor rats, and declined in the order sucrose-fed > chow-fed > polyunsaturated-fat-fed > starved. This was the case irrespective of the presence or absence of exogenous oleate. The contribution of newly synthesized fatty acids to the total mass of VLDL triacylglycerol also declined in the above order, and reflected the relative rates of fatty acid synthesis *de novo* in each of the groups. The contribution of exogenous oleate to VLDL triacylglycerol varied in a manner similar to that for newly synthesized fatty acid. However, the contribution either of exogenous oleate or of newly synthesized fatty acid never exceeded 17–20% of the total VLDL triacylglycerol fatty acid even in the sucrose-fed animals. The increased contribution of newly synthesized fatty acids in the sucrose-fed group was not sufficient to account for the increase in the total mass of VLDL triacylglycerol secreted. These results suggest that: (*a*) changes in the rate of triacylglycerol secretion are not a direct consequence of variations in the rate of fatty acid synthesis *de novo*; (*b*) in the short term, most of the triacylglycerol required for VLDL assembly and secretion is derived from an intracellular storage source; (*c*) the distribution of newly synthesized triacylglycerol between the cytosolic and secretory pools was similar irrespective of the source of fatty acids (i.e. synthesized *de novo* or exogenous).

INTRODUCTION

In vivo, several sources of fatty acid are potentially available for the synthesis of hepatic very-low-density lipoprotein (VLDL) triacylglycerol. These sources are: (*a*) endogenous synthesis from small precursors; (*b*) exogenous non-esterified fatty acid; (*c*) fatty acids derived from incoming lipoprotein triacylglycerol (e.g. remnants, low-density lipoprotein); (*d*) the intracellular triacylglycerol storage pool. In the intact rat, only a small proportion of the secreted VLDL triacylglycerol fatty acids (TGFA) are derived directly from the biosynthetic source [1,2], and this conclusion is supported by studies *in vitro* using perfused livers [3]. Little is known, however, of the effects of nutritional state on the capacity of the liver to utilize biosynthetic or extracellular fatty acids for VLDL triacylglycerol synthesis. For instance, large variations in the rate of hepatic fatty acid synthesis may be induced rapidly *in vitro* by supplementation with lipogenic substrates such as lactate and pyruvate [4–6]. Under these conditions, the low basal rates of fatty acid synthesis in hepatocytes from starved animals are restored to normal [5]. Information as to whether changes in nutritional state affect the ability of the liver cell to utilize this additional source of fatty acid for VLDL triacylglycerol synthesis is currently lacking.

Another question which remains to be resolved is whether the liver discriminates between fatty acids synthesized *de novo* and extracellularly pre-formed fatty acids as a source of substrate for VLDL triacylglycerol and, if so, whether this is influenced by nutritional state. In view of the complexity of the plasma fatty acid mixture available to the liver *in vivo*, it would have been impracticable to try to simulate this *in vitro*. Instead, in common with other investigators [3,7], we have used various concentrations of oleate as a representative exogenous fatty acid. The quantity of newly synthesized fatty acids available for VLDL triacylglycerol synthesis was varied by changing the concentrations of lactate and pyruvate in the medium [4–6]. Total carbon flux into fatty acids was measured by using ^3H_2O incorporation [8].

Finally, previous studies [9,10] have emphasized the importance of the cytosolic pool of triacylglycerol as a source of VLDL triacylglycerol. This, of course, is not a primary source, but the extent of its utilization for VLDL triacylglycerol synthesis will provide useful information about the quantity of newly synthesized and exogenous fatty acids which must pass through this pool before secretion. The present study has been designed to assess the contribution of this pool to VLDL triacylglycerol synthesis in different nutritional states.

MATERIALS AND METHODS

Maintenance of animals, hepatocyte preparations and incubation procedures

Male Wistar rats were housed in a windowless room artificially lit with tungsten bulbs between 16:00 h and 04:00 h. After at least 10 days the animals were assigned to one of four groups. One group was fed *ad libitum* with a chow diet (Diet 41B; Dixon and Sons, Ware, Herts., U.K. [2]). Another group was fed with a diet in which the chow was supplemented with 20% (w/w) corn oil [11]. A third group was fed with the chow diet and, in addition, was allowed unrestricted access to a solution of sucrose (25%, w/v) in the drinking water [12,13]. The sucrose- and fat-supplemented diets were given *ad libitum* for 7 days before hepatocyte preparation. The final group (starved rats) was fed with the chow diet *ad libitum*, and this was removed 24 h before hepatocyte preparation. The weights and food intakes of the rats used are given in Table 1. Hepatocytes were prepared at 10:00 h as described previously [14,15] and incubated in Krebs–Henseleit bicarbonate buffer supplemented with 3.5% (w/v) BSA, glucose (11.1 mM) and amino acids [16] in a total volume of 3.0 ml.

Abbreviations used: VLDL, very-low-density lipoprotein; ER, endoplasmic reticulum; TGFA, triacylglycerol fatty acids.

Table 1. Weights and food consumption of rats

Rats were weighed before and after a period of 7 days.

Diet	Final weight (g)	Weight gain (g)	Food intake (g/day)	Sucrose consumption (g/day)
Chow	248±7	45±6	25±1	–
Fat	259±21	28±5	21±1	–
Starved	207±11	28±3	25±1	–
Sucrose	231±5	39±1	17±1	8.1±0.4

Where appropriate, mixtures of [U-^{14}C]lactate and unlabelled pyruvate (10:1 ratio) were added to give initial lactate concentrations of 0 (tracer only), 2, 5 and 10 mM. The lactate specific radioactivities 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 the above concentrations of [^{14}C]lactate. This information was then used to calculate the contribution of fatty acid, synthesized from all sources of carbon, to cellular and VLDL TGFA (see below). In other incubations [^{3}H]oleate (1.33 μCi/μmol) was present at concentrations of 0.25, 0.50 and 0.75 mM. Incubations were carried out for 3 h, during which time the rates of secretion of VLDL and the synthesis of fatty acids did not decline [6,14]. The initial viabilities of the cells (measured by Trypan Blue exclusion) were 90.3±1.3%, 91.8±1.2%, 93.9±1.9% and 95.5±0.3% for the chow-fed, fat-fed, starved and sucrose-fed groups respectively. Viabilities did not decrease significantly after 3 h in either the absence (90.6±1.0%) or the presence (89.5±1.9%) of 0.75 mM-oleate.

Isolation of VLDL and cellular TGFA

After 3 h incubation, the cell pellet was removed by sedimentation and the VLDL was isolated from the cell supernatant [17]. The cellular and VLDL lipids were extracted [18]. A sample of each lipid extract was used for measurement of the total mass of triacylglycerol with a kit supplied by Boehringer Mannheim U.K. (Lewes, Sussex, U.K.). Triacylglycerol was purified from the remaining lipid extract by t.l.c., and the fatty acids were obtained after hydrolysis and solvent extraction [11]. Manipulative losses of TGFA 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.

Calculation of the contribution of newly synthesized fatty acids to cellular and VLDL triacylglycerol

The total mass and ^{14}C specific radioactivities of fatty acids newly synthesized in the presence of various concentrations of [^{14}C]lactate was determined by carrying out incubations identical with those used for isolation of VLDL and cellular triacylglycerol. In these cases, however, ^3H_2O (26.4 d.p.m./nmol) was simultaneously present at each concentration of [^{14}C]lactate. At the end of the incubation the saponifiable fraction of the cell (containing the labelled fatty acids) was isolated. From the relationship determined by Jungas [8], 1 μmol of C_{18} fatty acid synthesized from all sources of carbon incorporates 10.34 μmol of ^3H_2O. Thus we were able to calculate the total mass (nmol) of newly synthesized fatty acids. At each concentration of [^{14}C]lactate, the ^{14}C specific radioactivity of the newly synthesized fatty acids (d.p.m./nmol) was calculated. This factor was then used to convert the ^{14}C radioactivities of the VLDL and cellular TGFA into the total mass (nmol) of newly synthesized fatty acids incorporated.

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.) [19] before addition to the hepatocyte suspensions. Corn oil was purchased locally, and its composition (by wt.) was probably similar to that reported previously (i.e. 50% linoleic, 34% oleic and 13% palmitic [11]).

RESULTS

Rates of VLDL triacylglycerol secretion

Hepatocytes from the sucrose-fed rats had the highest rates of VLDL-triacylglycerol secretion irrespective of the concentrations of lactate or oleate in the incubation medium (Fig. 1). Inclusion of polyunsaturated fat in the diet led to a decline in the output of triacylglycerol, and this relatively low secretion persisted even when the medium was supplemented with various concentrations of oleate. Of all the nutritional states, starvation resulted in the lowest amounts of triacylglycerol secreted during the 3 h period. As expected, increasing the concentration of oleate in the incubation medium stimulated triacylglycerol output in all groups, although these changes did not achieve statistical significance. The somewhat lower basal triacylglycerol output in the oleate-containing series was most probably due to the increased albumin content of the incubation medium [20] compared with those containing lactate.

Contribution of exogenous and endogenous fatty acids

In the absence of lactate, the contribution of newly synthesized fatty acids as a percentage of the total VLDL TGFA decreased in the order sucrose-fed > chow-fed > fat-fed > starved (Fig. 2). Increasing the lactate concentration of the medium stimulated fatty acid synthesis (Fig. 4) and, in each group, led to an increase in the proportion of the secreted TGFA derived from this source. Nevertheless, the relative effect of nutritional state on the contribution of newly synthesized fatty acids remained unchanged

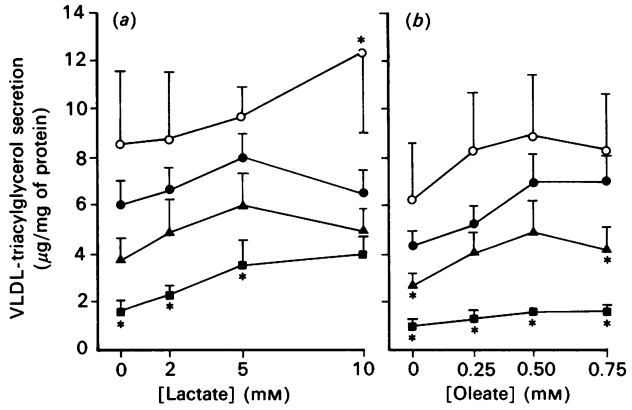

Fig. 1. Effect of nutritional state on the secretion of VLDL triacylglycerol

Hepatocytes were incubated for 3 h in the presence of various concentrations of lactate (*a*), to increase the supply of biosynthetic fatty acids, or of oleate (*b*), to increase that of exogenous fatty acids. The VLDL fraction was isolated and its triacylglycerol content determined. Each point represents the mean±S.E.M. of the values obtained from 10 chow-fed rats (●), 9 fat-fed rats (▲), 4 starved rats (■) and 4 sucrose-fed rats (○). Values marked * are significantly different ($P < 0.05$) from the corresponding values in the chow-fed controls.

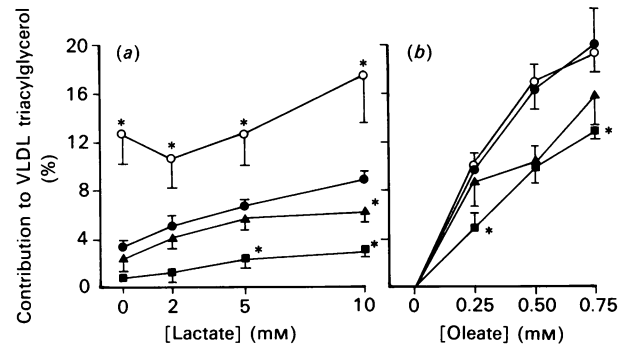

Fig. 2. Contribution of newly synthesized (*a*) and exogenous (*b*) fatty acids to VLDL triacylglycerol

Hepatocytes were incubated for 3 h with [^{3}H]oleate or [^{14}C]lactate. When [^{14}C]lactate was used, parallel incubations were carried out with ^3H_2O (26.4 d.p.m./nmol) to determine the total mass of newly synthesized fatty acids and their ^{14}C specific radioactivity. The mass of newly synthesized fatty acid incorporated into VLDL TGFA was then calculated from the ^{14}C radioactivity incorporated and the ^{14}C specific radioactivity of the newly synthesized fatty acids. Key: ●, chow-fed ($n = 6$); ▲, polyunsaturated-fat-fed ($n = 4$); ■, 24 h-starved ($n = 4$); ○, sucrose-fed ($n = 4$). Values marked * are significantly different ($P < 0.05$) from the corresponding values in the chow-fed controls.

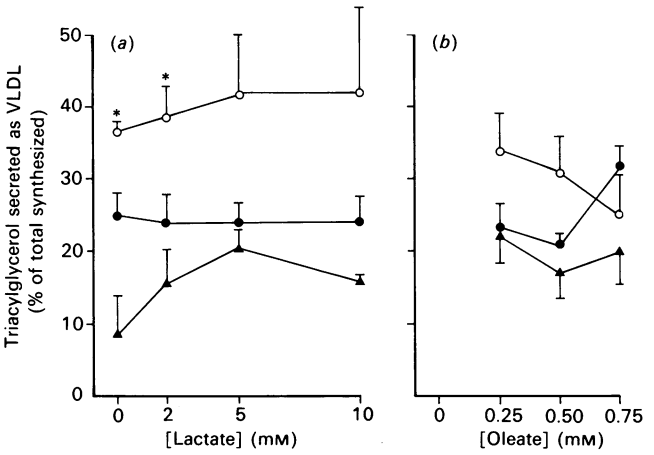

Fig. 3. Secretion and cellular retention of newly synthesized triacylglycerol

Hepatocytes were incubated for 3 h with various concentrations of [^{14}C]lactate (*a*) or [^{3}H]oleate (*b*). At the end of this period the VLDL and cellular triacylglycerol fractions were obtained and the radioactivity of their component fatty acids was determined. The mass of newly synthesized fatty acid incorporated was determined as described in the legend to Fig. 2. Key: ●, chow-fed ($n = 5$); ▲, polyunsaturated-fat-fed ($n = 4$); ○, sucrose-fed ($n = 3$). Values marked * are significantly different ($P < 0.05$) from the corresponding values in the chow-fed controls.

at each lactate concentration (Fig. 2). In general, the percentage contribution to the secreted TGFA from this source was very low (Fig. 2), and even at the highest observed rate of fatty acid synthesis (hepatocytes from sucrose-fed rats incubated with 10 mM-lactate) this amounted to only 17.5% of the totalTGFA secreted. The percentage contribution of exogenous oleate to VLDL TGFA also varied according to the nutritional state of the donor animals. This pattern was similar to that observed for the newly synthesized fatty acids, except that the relative contributions were similar in the sucrose-fed and chow-fed groups

Table 2. Distribution of newly synthesized TGFA

Hepatocytes from chow-fed, polyunsaturated-fat-fed and sucrose-fed rats were incubated in the presence of [^{14}C]lactate (10.0 mM) plus pyruvate (1 mM), or [^{3}H]oleate (0.25 mM and 0.75 mM). In each case, separate incubations were set up identical with those containing [^{14}C]lactate, except that ^3H_2O was present to determine the ^{14}C specific radioactivities of the newly synthesized fatty acids. After 3 h, the VLDL and cellular triacylglycerol fractions were isolated and the radioactivities of their component fatty acids determined. Each value represents the mean ± S.E.M. from four rats (chow-fed and fat-fed) or three rats (sucrose-fed).

Nutritional state	Substrate	Newly synthesized TGFA (nmol/mg of protein) Total	VLDL	Cellular
Chow-fed	10 mM-lactate	13.4±2.8	3.1±0.7	10.3±2.3
	0.25 mM-oleate	6.1±0.7	1.5±0.4	4.6±0.5
	0.75 mM-oleate	14.7±3.8	4.7±1.4	10.0±2.3
Fat-fed	10 mM-lactate	5.7±1.2	0.9±0.2	4.8±1.0
	0.25 mM-oleate	3.5±0.2	0.8±0.1	2.7±0.2
	0.75 mM-oleate	12.4±1.3	2.3±0.3	10.1±1.6
Sucrose-fed	10 mM-lactate	25.7±6.4	11.9±5.4	13.7±1.8
	0.25 mM-oleate	7.7±3.4	2.7±1.2	5.0±2.2
	0.75 mM-oleate	20.7±9.9	5.0±2.2	15.7±7.8

(Fig. 2). Within each group, increasing the concentration of oleate increased its relative contribution to the total TGFA secreted. At each concentration, however, the initial pattern imprinted by nutritional state remained unchanged. It therefore appeared that nutritional states which led to an increase in fatty acid synthesis (Fig. 4) increased the capacity of the hepatocyte to utilize exogenous fatty acids for VLDL synthesis.

Secretion and cellular retention of newly synthesized triacylglycerol

In some experiments, the amounts of labelled triacylglycerol which remained within the cell were also determined in addition to the amounts secreted as VLDL. In each incubation the sum of label associated with the cell and that secreted into the medium was a measure of total cellular triacylglycerol synthesis. The effects of feeding diets rich in sucrose or in polyunsaturated fat on the distribution of TGFA newly synthesized from the exogenous or the endogenous source were studied in this series of experiments (Fig. 3). In addition to increasing the rate of fatty acid biosynthesis (Fig. 4) and the total production of triacylglycerol from this source (Table 2), sucrose feeding increased the proportion of the total which appeared as VLDL and decreased the proportion which remained within the cell compared with the chow-fed animals (Table 2). The addition of lactate to the medium, although stimulating the total amount of TGFA synthesized, had no effect on its distribution between the cellular and secreted triacylglycerol (Fig. 3). At all concentrations of lactate, the sucrose-fed group retained its ability to secrete a higher proportion of newly synthesized triacylglycerol compared with the chow-fed group. Feeding the polyunsaturated-fat diet, on the other hand, decreased both the total quantity of triacylglycerol synthesized from the endogenous source of fatty acid (Table 2) and the proportion of this total which was secreted as VLDL (Fig. 3). This pattern was retained irrespective of the concentration of lactate in the medium. A similar, but less pronounced, pattern emerged when exogenous oleate was the source for cellular triacylglycerol synthesis (Fig. 3). Increased cellular retention of newly synthesized triacylglycerol may con-

Table 3. Specific radioactivities of cellular and VLDL

Hepatocytes were incubated as described in the legend to Table 2. Portions of the total lipid extracts derived from the cells and from the VLDL were used for enzymic assay of triacylglycerol mass. Triacylglycerol was purified from the remainder by t.l.c., and the radioactivity of the component fatty acids was determined. Each value represents the mean ± S.E.M. from four rats (chow-fed and fat-fed) or three rats (sucrose-fed).

Nutritional state	Substrate	TGFA sp. radioactivity (d.p.m./nmol)	
		Cellular	VLDL
Chow-fed	10 mM-lactate	29.7 ± 8.9	68.5 ± 15.2
	0.25 mM-oleate	550 ± 125	1057 ± 213
	0.75 mM-oleate	947 ± 241	2062 ± 389
Fat-fed	10 mM-lactate	8.2 ± 2.0	30.1 ± 5.4
	0.25 mM-oleate	231 ± 76.0	1105 ± 519
	0.75 mM-oleate	709 ± 95	1953 ± 454
Sucrose-fed	10 mM-lactate	75.6 ± 24.3	197 ± 114
	0.25 mM-oleate	501 ± 169	1219 ± 162
	0.75 mM-oleate	1306 ± 386	2349 ± 433

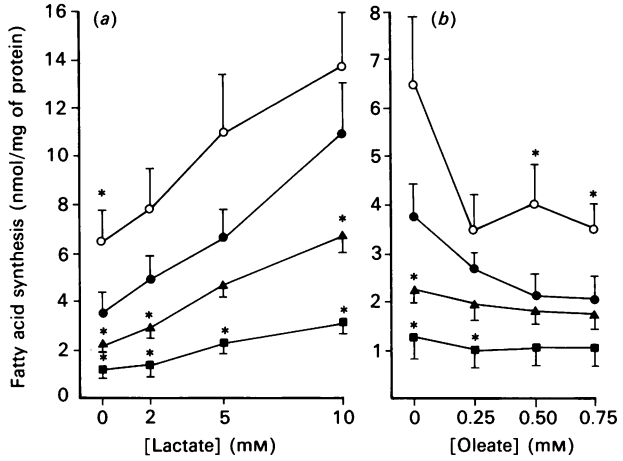

Fig. 4. Effect of nutritional state on the synthesis of fatty acids

Hepatocytes were incubated for 3 h in the presence of ^3H_2O (26.4 d.p.m./nmol) with various concentrations of lactate (*a*) or oleate (*b*). After this time the fatty acid fraction of the cell was obtained and its radioactivity determined. Key: ●, chow-fed ($n = 7$); ▲, polyunsaturated-fat-fed ($n = 6$); ■, 24 h-starved ($n = 4$); ○, sucrose-fed ($n = 4$). Values marked * are significantly different ($P < 0.05$) from those for chow-fed animals at the corresponding concentration of lactate or oleate.

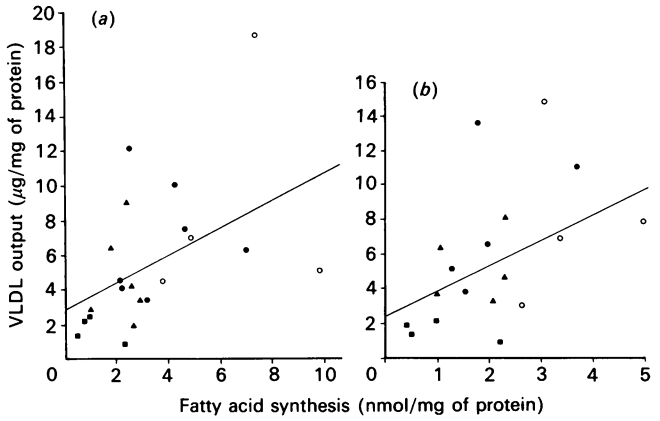

Fig. 5. Correlation between fatty acid synthesis and VLDL output in the presence and absence of exogenous oleate (0.75 mM)

Hepatocytes from chow-fed (●), polyunsaturated fat-fed (▲), 24 h-starved (■) and sucrose-fed (○) animals were incubated for 3 h in the presence (*b*) or absence (*a*) of 0.75 mM-oleate. At the end of this period the VLDL triacylglycerol mass was determined. Parallel incubations were conducted in the presence of ^3H_2O (26.4 d.p.m./nmol), and after 3 h the incorporation of ^3H into total cellular fatty acids was measured.

tribute to the increased total cellular triacylglycerol content of the hepatocytes from the fat-fed animals compared with the chow-fed controls (i.e. 60.3 ± 14.3 and 23.3 ± 3.8 nmol/mg of protein respectively).

In hepatocytes from the chow-fed animals, despite changes in the total amounts of triacylglycerol synthesized, the proportion of this total which was secreted as VLDL was the same irrespective of whether endogenous or exogenous fatty acids were used as the source of triacylglycerol. This proportion was about 25% in each case (Fig. 3). The same was true for the sucrose-fed animals (30–40%) in each case) and, apart from the incubation in the absence of lactate, in the fat-fed animals (16–22%). In all the nutritional states examined, irrespective of the source of the triacylglycerol precursor, the specific radioactivity of the VLDL TGFA was higher than that of the cellular material (Table 3).

Response of endogenous fatty acid synthesis to lactate and oleate: correlation with VLDL triacylglycerol secretion

In the absence of lactate, nutritional manipulation of the donor animals produced large variations in the rates of fatty acid synthesis *de novo* in the hepatocytes derived from them (Fig. 4). These rates increased in the order starved < fat-fed < chow-fed < sucrose-fed. Addition of lactate to the medium increased the rate in each group, but the overall relative pattern remained unchanged. The inhibitory effect of exogenous oleate appeared to be greater the higher the basal rate of fatty acid synthesis. Thus, at 0.25 mM-oleate, synthesis *de novo* was inhibited to a greater extent in the sucrose-fed group, followed by the chow-fed, the fat-fed and, finally, the starved. Oleate caused little or no inhibition of fatty acid synthesis in the last two groups. From the data displayed in Figs. 1 and 4 it is possible to attempt to correlate fatty acid synthesis rate with VLDL triacylglycerol output in hepatocytes from each individual liver. For incubations in the absence of oleate and lactate, by using data from all four groups, there was a positive correlation between the two variables ($r = 0.488$, $P = 0.025$; Fig. 5). This correlation persisted even when fatty acid synthesis rates were suppressed *in vitro* by addition of 0.75 mM-oleate to the medium ($r = 0.504$, $P = 0.033$).

DISCUSSION

It has been known for some time that starvation [21,22] and fat consumption [17,23] decrease, and sucrose consumption increases [12,13,21], VLDL secretion from isolated liver preparations. However, the effect of nutritional factors in determining the relative importance of fatty acids from various sources as precursors for VLDL triacylglycerol remains unclear. In rats fed on a normal chow diet, newly synthesized fatty acids comprise only a small proportion of the total VLDL triacylglycerol secreted

[3]. The present results show that, when fatty acid synthesis rates are suppressed by starvation or by feeding a high-polyunsaturated-fat diet, this source provides an even smaller proportion of the decreased total mass of secreted VLDL triacylglycerol. In the fat-fed animals, at least, this was due to an altered intracellular distribution of newly synthesized triacylglycerol such that a smaller proportion entered the secretory pool and a larger amount remained within the cell (Table 2). Both starvation [24] and fat consumption [17,25] are associated with increased plasma non-esterified fatty acid concentrations compared with normally fed rats. Increasing the availability of extracellular fatty acids *in vitro*, however, showed that the capacity of the cells from the starved and fat-fed animals to utilize fatty acids from this source remained lower than in the chow-fed group and resembled the pattern of utilization of endogenously synthesized fatty acids. Again, as was the case with endogenous fatty acids, fat-feeding resulted in an altered partitioning of triacylglycerol synthesized from exogenous oleate in favour of retention by, rather than secretion from, the cell (Fig. 3, Table 2). The cause of this change is not known. It has been suggested, however, that the availability of functional apoprotein B in the leaflet of the endoplasmic-reticulum membrane directs newly synthesized triacylglycerol into the endoplasmic-reticulum lumen for secretion as VLDL rather than into the cytosolic storage pool [26]. In this respect, a high dietary fat content either during the suckling period [27,28] or in adults [29] resulted in a decreased synthesis and secretion of apoprotein B.

In hepatocytes from sucrose-fed animals, despite the high rates of fatty acid synthesis (Fig. 4), during incubation in the absence of lactate, only $12.9 \pm 2.5\%$ of the VLDL triacylglycerol was derived from this source. This increased somewhat to $17.5 \pm 4.0\%$ in the presence of 10 mM-lactate. Nevertheless, this represented between 40 and 50% of the total cellular triacylglycerol newly synthesized from endogenously produced fatty acids (Fig. 3 and Tabie 2). From the data in Table 2, it may be calculated that in the sucrose-fed rats newly synthesized fatty acids contributed an extra 8.8 nmol to the total VLDL TGFA (i.e. 11.9 and 3.1 nmol in the sucrose-fed and control animals respectively). This is equivalent to 2.9 nmol of triacylglycerol. However, at this concentration of lactate, the sucrose-fed hepatocytes secreted a total of 6.3 nmol of VLDL triacylglycerol more than the controls (Fig. 1). Clearly, therefore, in the sucrose-fed group, the increased contribution of newly synthesized fatty acids was not sufficient to account for the increased VLDL triacylglycerol secreted by hepatocytes from these animals. In this respect, Ontko and his colleagues [30] have previously demonstrated an increased mobilization of intracellular lipid stores for VLDL triacylglycerol synthesis in sucrose-fed rats. Rates of hepatic lipogenesis are very high when fasted rats are re-fed with a high-carbohydrate diet. Although we did not specifically study this aspect, it is possible that, under these conditions, newly synthesized fatty acids play a more prominent role in contributing to VLDL triacylglycerol.

The low contribution of newly synthesized triacylglycerol to the total secreted triacylglycerol suggests that, in the short term, unlabelled cellular glycerolipid contributes the major proportion of the VLDL triacylglycerol. This is not a primary source and, *in vivo*, is derived from newly synthesized fatty acids, plasma non-esterified fatty acids and fatty acids derived from incoming lipoprotein triacylglycerol. Mobilization of this intracellular glycerolipid for VLDL assembly requires lipolysis followed by re-esterification [3,9,10]. Regulation of this lipolytic step may therefore play a major role in the control of VLDL triacylglycerol secretion. In this regard, we have previously shown that insulin suppresses the mobilization of cytosolic triacylglycerol [31], and this is paralleled by a decline in the secretion of VLDL triacylglycerol [31] and apoB [32,33].

The data in Table 3 suggest that a proportion of the newly synthesized triacylglycerol does not equilibrate with the cellular pool, but is secreted directly as VLDL. This implies that a portion of the triacylglycerol newly synthesized at the cytosolic face of the endoplasmic reticulum [34] enters the secretory apparatus directly for assembly into VLDL and another portion enters the cell cytosol for storage. In each group of animals, the relative difference in specific radioactivity between the VLDL and cellular triacylglycerol was the same for exogenous oleate as for newly synthesized fatty acids. This suggests that factors which determine partitioning between VLDL and cytosol do not discriminate between triacylglycerol synthesized from exogenous and endogenous sources. Further support for this comes from the data in Fig. 3, which show that, in any given nutritional state, of the total newly synthesized triacylglycerol, similar proportions are secreted as VLDL irrespective of whether exogenous or endogenous fatty acids were used as the triacylglycerol precursor.

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