Regulation of hepatic very-low-density lipoprotein secretion in rats fed on a diet high in unsaturated fat

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Rats were fed *ad libitum* on either a standard, high-carbohydrate, chow diet or a similar diet supplemented with 15% unsaturated fat (corn oil). Hepatocytes were prepared either during the dark phase (D_6 -hepatocytes) or during the light phase (L_2 -hepatocytes) of the diurnal cycle. In hepatocytes from rats fed on the unsaturated-fat-containing diet, secretion of very-low-density lipoprotein (VLDL) triacylglycerol was inhibited to a greater extent in the D_6 - than in the L_2 -hepatocytes. Plasma non-esterified fatty acid concentrations were elevated to the same extent at both D_6 and L_2 in the unsaturated-fat-fed animals. The secretion of VLDL esterified and non-esterified cholesterol was relatively insensitive to changes in the unsaturated-fat content of the diet. This resulted in proportionate increases in the content of these lipid constituents compared with that of triacylglycerol in the nascent VLDL. There was also an increase in the ratio of esterified to non-esterified cholesterol in the nascent VLDL produced by hepatocytes of the unsaturated-fat-fed animals. In the D_6 -hepatocytes from the unsaturated-fat-fed animals, the decrease in the secretion of VLDL triacylglycerol could not be reversed by addition of exogenous oleate (0.7 mM) to the incubation medium. In contrast, addition of a mixture of lactate (10 mM) and pyruvate (1 mM) stimulated both fatty acid synthesis *de novo* and the rate of VLDL triacylglycerol secretion. Secretion of esterified and non-esterified cholesterol also increased under these conditions. Insulin suppressed the secretion of VLDL triacylglycerol and cholesteryl ester under a wide range of conditions in all types of hepatocyte preparations. Non-esterified cholesterol secretion was unaffected. In hepatocytes prepared from the fat-fed animals, these effects of insulin were more pronounced at D_6 than at L_2 . Glucagon also inhibited VLDL lipid secretion in all types of hepatocyte preparations. The decrease in cholesterol secretion was due equally to decreases in the rates of secretion of both esterified and non-esterified cholesterol.

INTRODUCTION

It is well established that, in isolated liver preparations from animals fed on a high-carbohydrate diet, increasing the availability of exogenous fatty acids *in vitro* stimulates the rate of secretion of triacylglycerol associated with VLDL (Goh & Heimberg, 1979; Topping & Mayes, 1982; Davis & Boogaerts, 1982). Paradoxically, however, the increased plasma NEFA resulting from consumption of fat-enriched diets (Heimberg *et al.*, 1974) appear, in some cases, to be associated with a decrease in hepatic VLDL triacylglycerol secretion (Kalopissis *et al.*, 1981; Wong *et al.*, 1984). Although diets high in certain types of fat are known to change the hormonal composition of the serum (Raboli & Martin, 1977; Weekes *et al.*, 1986), little is known of the possible hormonal control of hepatic VLDL metabolism under these conditions. One of the objectives of the present study was to investigate the effects of insulin and glucagon on VLDL secretion in hepatocytes prepared from animals fed on a diet enriched in polyunsaturated fatty acid (corn oil).

Pronounced diurnal variations in hepatic lipid metabolism occur both in experimental animals (see, e.g.,

Hems *et al.*, 1975; Gibbons *et al.*, 1984) and in man (Schlierf & Dorow, 1973; Parker *et al.*, 1982), probably as a result of the rhythmic changes in food consumption. In view of this, we wished to determine whether diurnal changes in food intake modified the response of hepatic VLDL secretion to diets high in polyunsaturated fat.

Finally, although the relationship between dietary fat intake and plasma lipoprotein composition has been under intense scrutiny for several years (see, e.g., Goldberg & Schonfeld, 1985), no information is available concerning the effect of high-fat-containing diets on the secretion of hepatic VLDL esterified and non-esterified cholesterol. Of particular interest in this regard is the possibility of diet-induced changes in the relative lipid content of the nascent VLDL, since changes of this type are known to affect the subsequent metabolism of VLDL (Demel & Jackson, 1985; O'Looney *et al.*, 1985) and of its product, LDL (Lopes-Virella *et al.*, 1982; Goldstein & Brown, 1984; Hiramatsu *et al.*, 1985). For these reasons we considered it important to determine the precise effects of the unsaturated-fat-supplemented diet on the relative amounts of various lipids in ratios of the newly secreted VLDL.

Abbreviations used: VLDL, very-low-density lipoprotein; LDL, low-density lipoprotein; NEFA, non-esterified fatty acids.

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MATERIALS AND METHODS

Maintenance of animals, preparation of hepatocytes and incubation conditions

Male Wistar rats were housed in windowless rooms, which were artificially lit with tungsten bulbs for a continuous period of 12 h in each day. Groups of rats were fed on a commercially available pelleted diet (Diet PRM; E. Dixon and Sons, Ware, Herts, U.K.), which contained (w/w) 47.5% carbohydrate (mainly starch), 2.7% fat and 18.7% protein. Food and water were available *ad libitum*. Other groups of rats were fed on the above diet supplemented with corn oil (15%, w/w) (Gibbons & Pullinger, 1986). Animals were fed on this diet for 7 days before preparation of hepatocytes. During this time there were no significant differences in food consumption, weight gain or final weight between animals maintained on each type of diet (Gibbons & Pullinger, 1986).

Hepatocytes were prepared from animals weighing between 230 and 300 g at a time mid-way through the 12 h dark phase of the light/dark cycle (D_6 -hepatocytes) or at a point 2 h into the light phase of the cycle (L_2 -hepatocytes). Procedures for the preparation and incubation of hepatocytes have been described previously (Seglen, 1976; Pullinger & Gibbons, 1983, 1985). The standard incubation medium contained Krebs-Henseleit (1932) bicarbonate buffer, to which was added Ca^{2+} (2.5 mM), glucose (11.1 mM), amino acids (East *et al.*, 1973) and fatty acid-free bovine serum albumin (3.6%, w/v). Where appropriate, a solution of a mixture of lactate and pyruvate was prepared in incubation medium and added to the flasks to give initial concentrations of 10 mM and 1 mM respectively (Boyd *et al.*, 1981). Oleate was complexed with albumin (Van Harken *et al.*, 1969) and added to the incubation flasks to give an initial concentration of 0.7 mM. In some cases, glucagon or insulin was added, each as a solution in 0.9% NaCl, to give initial concentrations of 0.1 μ M and 1 munit/ml respectively. To compensate for hormone biodegradation (McGarry *et al.*, 1978; Beynen *et al.*, 1979), similar quantities of each hormone were added at 1 h intervals. In those experiments in which the rate of fatty acid synthesis was determined, 3H_2O (1 mCi/ml) was present in the incubation medium and the rate of synthesis was measured as described previously (Gibbons *et al.*, 1984).

Isolation of secreted VLDL and measurement of associated triacylglycerol, esterified and non-esterified cholesterol

At the end of the incubation period, the cell suspension (2.0 ml) was chilled on ice and the supernatant was obtained by centrifugation at 80 g for 1.5 min. The supernatant fraction was further centrifuged at 20000 g and the volume adjusted to 5.0 ml with a solution of d 1.006 (Havel *et al.*, 1955). The fraction of d < 1.006 (containing the VLDL) was obtained by centrifugation in a Beckman SW 50.1 rotor at 40000 rev./min for 16 h. The top 1.0 ml of the tube contents (containing the VLDL fraction of d < 1.006) was obtained by slicing.

The total (esterified plus non-esterified) cholesterol was determined by treatment of a sample of the d < 1.006 fraction with cholesterol oxidase and cholesterol esterase. This converted both the esterified and non-esterified cholesterol into cholest-4-en-3-one, which was then assayed by g.l.c. with electron-capture detection

(Pullinger & Gibbons, 1985). Non-esterified cholesterol was determined in a similar sample by using an identical method except that treatment with cholesterol esterase was omitted. The amount of esterified cholesterol was determined by subtracting the amount of non-esterified cholesterol from the total.

Triacylglycerol was measured in a further portion of the d < 1.006 fraction by the glycerophosphate oxidase method (Trinder, 1969).

Other analytical methods

Protein was determined by the method of Gieger & Bessman (1972). For determination of plasma non-esterified fatty acids, a blood sample was taken from the descending vena cava into a heparinized syringe. The plasma was obtained by centrifugation and the non-esterified fatty acid content was determined (Shimizu *et al.*, 1979).

Statistical treatment of results

All values are presented as the means $\pm$ S.E.M. for several independent observations. The significance of any observed difference was tested by Student's *t* test.

Materials

The sources of materials used in this work have been described previously (Gibbons & Pullinger, 1979; Gibbons *et al.*, 1986). Radiochemicals were obtained from Amersham International, Little Chalfont, Bucks, U.K. Corn oil was purchased locally and its fatty acid composition was probably similar to that reported previously (Ide *et al.*, 1978). The triacylglycerol assay kit was obtained from the Boehringer Corp. (London).

RESULTS AND DISCUSSION

Diurnal variation in the effects of a high-unsaturated-fat diet on the secretion of hepatic VLDL triacylglycerol, esterified and non-esterified cholesterol

Hepatocytes were prepared at D_6 , the time of maximum food consumption (Fukuda *et al.*, 1985), from rats maintained either on a normal high-carbohydrate chow diet or on a similar diet supplemented with 15% (w/w) unsaturated fat. Immediately before cannulation of the portal vein, a blood sample was taken from the inferior vena cava and the concentration of the plasma NEFA was determined. The hepatocytes were incubated in the standard medium for 3 h, after which the cells were removed by centrifugation. The fraction of the supernatant containing the VLDL was isolated and the amounts of triacylglycerol, esterified and non-esterified cholesterol were determined. It has been shown previously that, under these conditions, the secretion of triacylglycerol and cholesterol does not decrease with time for at least 3 h (Pullinger & Gibbons, 1985). Similar experiments were carried out at L_2 , a time when food consumption was low (Fukuda *et al.*, 1985). The results are presented in Table 1, which shows that, irrespective of the time of day, consumption of the unsaturated-fat-containing diet gave a significant increase in the concentration of plasma NEFA. Nevertheless, at D_6 , despite the increased availability of this potential precursor of hepatic triacylglycerol, the secretion of VLDL triacylglycerol was decreased by about 65% (Table 1) (P < 0.05). At L_2 , the inhibitory effect of the

Table 1. Diurnal effects of an unsaturated-fat-supplemented diet on hepatocyte VLDL secretion and plasma NEFA concentrations

Hepatocyte suspensions (3.0 ml) were incubated for 3 h at 37 °C in the standard medium. The cells were removed by centrifugation and the $d < 1.006$ fraction was obtained from the supernatant. Samples of this fraction were removed for assay of triacylglycerol, esterified and non-esterified cholesterol. Numbers in parentheses represent the numbers of independent hepatocyte preparations within each group. Values marked * are significantly different ($P < 0.05$) from those in animals fed with normal diet.

Time of day	Type of diet	Secretion of VLDL ($\mu\text{g}/\text{mg}$ of protein)			Plasma NEFA concn. (mM)
		Triacylglycerol	Esterified cholesterol	Non-esterified cholesterol	
D ₆	Normal	6.35 $\pm$ 1.19 (4)	0.12 $\pm$ 0.02 (4)	0.22 $\pm$ 0.03 (4)	0.34 $\pm$ 0.04 (4)
	Unsaturated fat	2.28 $\pm$ 0.34 (12)*	0.11 $\pm$ 0.02 (6)	0.18 $\pm$ 0.03 (6)	0.49 $\pm$ 0.04 (4)*
L ₂	Normal	7.22 $\pm$ 0.67 (4)	0.10 $\pm$ 0.01 (4)	0.22 $\pm$ 0.02 (4)	0.34 $\pm$ 0.03 (6)
	Unsaturated fat	4.81 $\pm$ 0.58 (12)*	0.10 $\pm$ 0.02 (4)	0.15 $\pm$ 0.04 (6)	0.47 $\pm$ 0.04 (4)*

Table 2. Effect of exogenous oleate and of lipogenic precursors on the rate of VLDL secretion in D₆-hepatocytes from unsaturated-fat-fed animals

Hepatocytes were incubated for 3 h either alone or in the presence of oleate (0.7 mM) or a mixture of lactate (10 mM) and pyruvate (1 mM). In those instances where the rate of fatty acid synthesis was measured, $^3\text{H}_2\text{O}$ (3 mCi) was present. Numbers in parentheses refer to the numbers of independent determinations: N.D., not determined. Values marked ** and *** are significantly different from the control (no addition) at $P < 0.01$ and $P < 0.001$ respectively.

Additions to flask	Secretion of VLDL ($\mu\text{g}/\text{mg}$ of protein)		
	Triacylglycerol	Non-esterified cholesterol	Esterified cholesterol
–	3.07 $\pm$ 0.45 (5)	0.18 $\pm$ 0.03 (6)	0.11 $\pm$ 0.02 (6)
Oleate	2.67 $\pm$ 0.36 (5)	0.19 $\pm$ 0.02 (6)	0.13 $\pm$ 0.02 (6)
Lactate + pyruvate	6.04 $\pm$ 0.41 (6)***	0.31 $\pm$ 0.03 (6)**	0.16 $\pm$ 0.02 (6)

unsaturated-fat diet was much less pronounced. The secretion of VLDL esterified and non-esterified cholesterol was much less sensitive to the increase in dietary unsaturated fat than was that of triacylglycerol (Table 1).

It could be argued that the decrease in VLDL triacylglycerol secretion by the hepatocytes at D₆ resulted from a depletion of hepatic triacylglycerol caused by the absence of a suitable substrate (NEFA) in the incubation medium. This seemed unlikely, for two reasons. First, inclusion of exogenous oleate in the medium at a concentration of 0.7 mM failed to restore a normal rate of VLDL-triacylglycerol secretion in the D₆-hepatocytes prepared from the unsaturated-fat-fed animals (Table 2). Second, the large increase in the pool of hepatic triacylglycerol in the unsaturated-fat-fed rats at both D₆ and L₂ (K. Collins & G. F. Gibbons, unpublished work) would tend to protect against triacylglycerol depletion in the derived hepatocytes.

The stimulatory effects of the lipogenic precursors, pyruvate and lactate, on VLDL triacylglycerol secretion contrasted sharply with the inertness of exogenous, pre-formed, oleate (Table 2). Thus, in the D₆-hepatocytes, these precursors were able to restore the rate of triacylglycerol secretion to that characteristic of animals fed on the control diet. The rate of secretion of esterified and non-esterified cholesterol also increased under these conditions. It is therefore possible that VLDL secretion, at least in fat-fed animals, is dependent more on the

availability of endogenous fatty acids produced *de novo*, rather than on the availability of exogenous NEFA.

Effects of a high-unsaturated-fat diet on the relative lipid content of newly secreted VLDL

The relative lipid content of triacylglycerol-rich particles affects their rates of catabolism and thus influences their rates of clearance from the plasma. Differences are observed not only in the rate of triacylglycerol hydrolysis by lipoprotein lipase (EC 3.1.1.34) (Demel & Jackson, 1985; O'Looney *et al.*, 1985) but also in the rate of receptor-mediated clearance of the ultimate product, LDL (Lopes-Virella *et al.*, 1982; Hiramatsu *et al.*, 1985; Witztum *et al.*, 1985). In those animal species in which protein-mediated cholesterol ester exchange occurs, the rate of this process is also influenced by changes in the relative content of non-esterified cholesterol in VLDL (Fielding *et al.*, 1982, 1984). In view of this, it was considered important, in the present work, to establish whether dietary supplementation with unsaturated fat produced changes in the amounts of triacylglycerol, esterified and non-esterified cholesterol, relative to each other. These results are shown in Table 3. At D₆, the relative insensitivity of total (esterified plus non-esterified) cholesterol secretion to inhibition by unsaturated-fat feeding gave rise to a 2-fold increase in the proportion of cholesterol relative to triacylglycerol. A similar but less pronounced change

Table 3. Effects of an unsaturated-fat-supplemented diet on the relative lipid content of VLDL secreted by hepatocytes

Hepatocytes were incubated for 3 h. For other details see the legend to Table 1.

Time of day	Type of diet	Non-esterified plus esterified cholesterol (% of triacylglycerol)	Cholesteryl ester (% of triacylglycerol)	Cholesteryl ester (% of non-esterified cholesterol)
D ₆	Normal	6.8 ± 1.4 (5)	2.8 ± 0.04 (3)	49 ± 11 (3)
	Unsaturated fat	13.3 ± 2.2 (12)	4.3 ± 1.00 (6)	62 ± 8 (6)
L ₂	Normal	4.4 ± 0.3 (4)	1.4 ± 0.16 (4)	48 ± 7 (4)
	Unsaturated fat	6.3 ± 1.0 (12)	3.2 ± 1.3 (4)	90 ± 18 (4)

was also observed at L₂. The high-unsaturated-fat diet also led to a significant increase in the content of cholesteryl ester relative to triacylglycerol. Combination of the values for hepatocytes prepared at L₂ and D₆ showed that this factor increased from $2.00 \pm 0.32\%$ ($n = 7$) in the control animals to $3.84 \pm 0.79\%$ ($n = 10$) ($P < 0.05$) in those fed on the unsaturated-fat-supplemented diet. Demel & Jackson (1985) have suggested that the cholesteryl ester/triacylglycerol ratio of triacylglycerol-rich lipoproteins may have an important influence on their rates of hydrolysis by lipoprotein lipase. If this is correct, then the type of change demonstrated in the present work may contribute to the means by which dietary unsaturated fat affects plasma VLDL concentration.

Changes were also observed in the content of esterified compared with non-esterified cholesterol in the nascent VLDL particle (Table 3). Again, combination of values for hepatocytes prepared at L₂ and D₆ showed that fat-feeding increased the amount of cholesteryl ester compared with non-esterified cholesterol from $48.0 \pm 5.5\%$ ($n = 7$) in animals fed on the control diet to $73.0 \pm 9.0\%$ ($n = 10$) ($P < 0.05$) in those receiving the unsaturated-fat supplement. If a similar change occurred in human subjects, this may affect the inter-lipoprotein transfer of cholesteryl ester, an important factor in the rate of clearance of the plasma cholesterol (Fielding *et al.*, 1982, 1984).

Effects of insulin and glucagon on the secretion of VLDL-associated triacylglycerol, esterified and non-esterified cholesterol

Previous work has shown that insulin inhibits the secretion of VLDL-associated triacylglycerol (Durrington *et al.*, 1982; Patsch *et al.*, 1983; Pullinger & Gibbons, 1985; Mangiapane & Brindley, 1986), apolipoproteins E and B and phospholipid (Patsch *et al.*, 1983) and total cholesterol (Pullinger & Gibbons, 1985) in isolated hepatocyte preparations. Similar effects have been observed in the secretion of VLDL-triacylglycerol in perfused animal liver (Nikkilä, 1974) and in human subjects *in vivo* (Pietri *et al.*, 1983). The physiological significance of this inhibitory effect of insulin has been discussed previously (Gibbons, 1986). All the above investigations with isolated liver preparations were carried out on donor animals which had been maintained on diets high in carbohydrate and low in fat. Little or nothing is known of the hormonal control of hepatic lipoprotein secretion in animals fed on diets with fat contents more similar to those of many human diets. Neither is there any information available on the effects

of peptide hormones on the relative lipid content of triacylglycerol, cholesteryl ester and non-esterified cholesterol. It has been noted above that this factor may be an important determinant of lipoprotein catabolism.

In the present work, these aspects were investigated by comparing the effects of insulin and glucagon on the secretion of these individual lipids in hepatocytes prepared from normal and from unsaturated-fat-fed animals. To account for possible diurnal variations in these effects, hepatocytes were prepared at either L₂ or D₆ in each case. The results are shown in Table 4. Insulin produced decreases in the rates of secretion of total (esterified plus non-esterified) cholesterol and of triacylglycerol in the control hepatocytes. However, unexpectedly, the decrease in total cholesterol output was due entirely to a decline in the secretion of esterified cholesterol. Insulin had no significant effect on the amounts of non-esterified cholesterol associated with the newly secreted VLDL.

Hepatocytes from the unsaturated-fat-fed animals responded to insulin in a similar manner. In this case, however, there was a tendency towards a greater inhibitory response in those hepatocytes prepared at D₆, compared with that in the control cells prepared at this time. Again, in the unsaturated-fat series, insulin also appeared to be somewhat more inhibitory at D₆ than at L₂, especially with regard to the secretion of cholesteryl ester (Table 4).

Glucagon inhibited the secretion of VLDL triacylglycerol to a greater extent than did insulin (Table 4). This was the case irrespective of whether the donor animals were fed on the normal or the unsaturated-fat-containing diet, or whether the hepatocytes were prepared at L₂ or D₆. In contrast with insulin, glucagon resulted in a decrease in the rate of secretion of non-esterified, as well as of esterified, cholesterol. The relative extents of these changes were similar in hepatocytes from both the control and the unsaturated-fat-fed animals (Table 4).

The differing susceptibility of secretion rate of each of the constituent lipids of VLDL to inhibition by glucagon or insulin resulted in changes in the relative lipid composition of the newly secreted VLDL when hepatocytes were incubated with these hormones. This was particularly well illustrated by the effect of insulin on the ratio of esterified:non-esterified cholesterol (Table 5). In hepatocytes from both the normal and the unsaturated-fat-fed animals, insulin resulted in significant ($P < 0.01$ and $P < 0.05$ respectively) decreases in this ratio, a change which, again, may have important implications for the metabolism and clearance of lipoprotein cholesterol (Fielding *et al.*, 1982, 1984). Glucagon, but

Table 4. Effects of insulin and glucagon on the secretion of VLDL by hepatocytes from normal and unsaturated-fat-fed animals

Hepatocytes were prepared at D₆ and L₂ from animals fed on either the normal chow diet or a similar diet supplemented with 15% unsaturated fat. The hepatocytes were incubated for 3 h in the presence or absence of insulin (1 munit/ml) or glucagon (0.1 μ M). The amounts of esterified and non-esterified cholesterol secreted with the VLDL fraction were determined in each case. The effects of insulin and glucagon are expressed as a percentage of the rate of secretion observed in their absence. The numbers in parentheses represent the numbers of independent hepatocyte preparations. In each category, the amounts of triacylglycerol, esterified and non-esterified cholesterol secreted in the control incubations are given in Table 1. The amounts of total cholesterol secreted in each control incubation (μ g/mg of protein) were as follows: D₆, normal 0.34 ± 0.02 ; D₆, unsaturated fat, 0.25 ± 0.03 ; L₂, normal, 0.31 ± 0.02 ; L₂, unsaturated fat, 0.26 ± 0.03 . Values marked *, ** and *** are significantly different from the controls at $P < 0.05$, $P < 0.01$ and $P < 0.001$ respectively.

Time of day	Type of diet	Additions to flask	Secretion of VLDL (% of control)			
			Triacylglycerol	Total cholesterol	Non-esterified cholesterol	Esterified cholesterol
D ₆	Normal	Insulin	86.3 $\pm$ 9.3 (4)	90.0 $\pm$ 9.4 (6)	113.5 $\pm$ 11.2 (4)	64.7 $\pm$ 15.9 (4)
		Glucagon	15.2 $\pm$ 9.0 (4)**	56.6 $\pm$ 5.9 (6)***	60.2 $\pm$ 6.3 (4)***	54.1 $\pm$ 7.6 (4)***
	Unsaturated fat	Insulin	81.6 $\pm$ 6.0 (12)**	80.4 $\pm$ 4.7 (12)**	90.7 $\pm$ 6.8 (6)	58.8 $\pm$ 14.6 (6)*
		Glucagon	35.4 $\pm$ 8.6 (12)***	55.2 $\pm$ 5.8 (12)***	51.8 $\pm$ 7.5 (6)***	57.0 $\pm$ 20.1 (6)
L ₂	Normal	Insulin	89.0 $\pm$ 8.2 (4)	87.1 $\pm$ 4.9 (4)	93.6 $\pm$ 4.8 (4)	69.7 $\pm$ 9.9 (4)*
		Glucagon	21.6 $\pm$ 7.8 (4)**	52.1 $\pm$ 3.6 (3)***	49.8 $\pm$ 1.9 (4)***	57.3 $\pm$ 6.7 (4)**
	Unsaturated fat	Insulin	86.2 $\pm$ 5.5 (12)*	90.1 $\pm$ 7.6 (11)	114.0 $\pm$ 24.9 (5)	90.7 $\pm$ 3.1 (4)*
		Glucagon	27.6 $\pm$ 5.1 (12)***	35.6 $\pm$ 5.2 (12)***	46.1 $\pm$ 10.7 (5)**	61.8 $\pm$ 4.8 (4)**

Table 5. Effect of hormones on the ratio of esterified to non-esterified cholesterol in newly secreted VLDL

Hepatocytes were incubated for 3 h. For other details see the legend to Table 1.

Type of diet	Incubation conditions				
	Control	Insulin	Effect of insulin (% of control)	Glucagon	Effect of glucagon (% of control)
Unsaturated fat	0.73 $\pm$ 0.09 (10)	0.56 $\pm$ 0.10 (10)	74.0 $\pm$ 10.1	0.83 $\pm$ 0.11 (10)	116.7 $\pm$ 18.1
Normal	0.48 $\pm$ 0.05 (7)	0.33 $\pm$ 0.07 (6)	62.7 $\pm$ 11.2	0.50 $\pm$ 0.06 (6)	107.8 $\pm$ 6.3

not insulin, also gave rise to an increase in the cholesteryl ester/triacylglycerol ratios in hepatocytes from both the normal and the unsaturated-fat-fed animals (results not shown).

General discussion

The nature of the mechanism(s) underlying the hypolipidaemic effects of diets high in unsaturated fats is currently the subject of intense scrutiny (Goldberg & Schonfeld, 1985). The present results implicate at least two operative factors. The first is a decreased flux of hepatic VLDL triacylglycerol into the plasma. The second is a possible change in the rate of clearance of hepatic VLDL and its metabolic products resulting from changes in the relative lipid content of the nascent particles.

The decreased rate of secretion of VLDL triacylglycerol in the presence of an increased quantity of substrate NEFA presents something of a paradox. The increased concentrations of hepatic triacylglycerol in the fat-fed animals suggest that there is no shortage of triacylglycerol available for secretion. In this regard, it is noteworthy that, in human subjects, consumption of a diet rich in ω -3 fatty acids leads to a decrease in the rate of synthesis of apolipoprotein B (Illingworth *et al.*,

1984). Since the availability of apolipoprotein B may limit VLDL secretion (Davis & Boogaerts, 1982), this may provide an explanation for the observed effects of the unsaturated-fat-containing diet.

In parallel with its effects on hepatic fatty acid and cholesterol synthesis (Gibbons & Pullinger, 1986), the high-unsaturated-fat diet also influenced hepatic VLDL to extents which varied with a circadian periodicity. Thus only at D₆, the time of maximum food consumption (Fukuda *et al.*, 1985), was there a large decrease in VLDL triacylglycerol secretion. At L₂, a time of low food (and thus low fat) consumption, the rate of VLDL triacylglycerol secretion rapidly reverted to that more characteristic of animals fed on a normal diet. Whatever the mechanism(s) underlying these rapidly reversible rhythmic effects of dietary unsaturated fat, the present results emphasize the importance of selecting the correct time of day in studying the dietary and, perhaps, the hormonal control of hepatic VLDL secretion.

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