Diurnal variations in the effects of an unsaturated-fat-containing diet on fatty acid and cholesterol synthesis in rat hepatocytes

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1. Rats were fed *ad libitum* on either a standard high-carbohydrate diet, or a standard diet supplemented with 15% corn oil. Hepatocytes were prepared either during the light phase (L₂-hepatocytes) or during the dark phase (D₆-hepatocytes) of the diurnal cycle. 2. In hepatocytes from rats fed on the fat-containing diet, fatty acid synthesis (lipogenesis) was suppressed to a much greater extent at D₆ than at L₂. The magnitude of the increase in plasma-free fatty acid concentration was similar at the two times of day. 3. The rate of cholesterol synthesis was also significantly suppressed in the D₆- but not in the L₂-hepatocytes. This differential inhibition resulted in the abolition of the normal diurnal rhythm of cholesterologenesis. The initial activity of 3-hydroxy-3-methylglutaryl-CoA reductase in hepatocytes was also suppressed by corn-oil feeding at D₆ but not at L₂. 4. In D₆-hepatocytes, the inhibitory effect of the high-fat diet on the conversion of lactate into cholesterol and fatty acids was greater than that on total carbon flux into these substances for all endogenous sources. Despite this, under these conditions a high concentration of lactate and pyruvate resulted in a several-fold stimulation of total carbon flux into fatty acids. 5. In hepatocytes prepared at L₂, fat-feeding had little effect on the degree of stimulation of lipogenesis by insulin or inhibition by glucagon. However, at D₆, fat-feeding blunted the response of lipogenesis to both these hormones.

INTRODUCTION

Most studies of the effects of insulin and glucagon on hepatic lipid synthesis have been carried out in animals fed on a diet high in carbohydrate (Geelen *et al.*, 1980). Although the relationship between dietary fat intake and hepatic lipid metabolism has been an active field of study for many years (see, e.g., Rosmos & Leveille, 1974; Triscari *et al.*, 1978; Jenke *et al.*, 1982; Abraham *et al.*, 1983; Baltzell & Berdanier, 1985), little is known concerning the response of hepatic lipogenesis to pancreatic hormones in animals consuming diets with higher contents of fat. This information may be of importance in understanding how changes in human diets, many of which have a high fat content, determine the exact response of hepatic lipogenesis to pancreatic hormones. The primary objective of this study, therefore, was to determine whether the direct response of lipogenesis to insulin and glucagon in isolated hepatocytes is modified by inclusion of unsaturated fat (corn oil) in the diet of the donor animals.

McGarry and his colleagues (Boyd *et al.*, 1981) have shown that the low rate of hepatic lipogenesis typical of the starved state *in vivo* persists in isolated hepatocytes obtained from animals deprived of food for various periods of time. Furthermore, this 'fasting' profile of lipogenesis may be rapidly reversed *in vitro* by incubating hepatocytes in the presence of suitable lipogenic substrates (Boyd *et al.*, 1981; Gibbons *et al.*, 1984). In addition to starvation, consumption of a diet high in unsaturated fat is also characterized by a decreased rate of hepatic lipogenesis *in vivo* (Triscari *et al.*, 1978; Abraham *et al.*, 1983). A further objective of the present study, therefore, was to determine whether this lipogenic pattern could also be rapidly reversed

in vitro by providing hepatocytes derived from fat-fed rats with a sufficient quantity of lipogenic precursors.

Finally, the rates of hepatic cholesterol synthesis (Edwards *et al.*, 1972; Rodwell *et al.*, 1976; Gibbons *et al.*, 1983) and lipogenesis (Scott & Potter, 1970; Hems *et al.*, 1975; Munday & Williamson, 1983) vary with a diurnal periodicity which appears to be dependent on the changing pattern of food intake. Previous work has shown that the magnitude of the lipogenic response to insulin, glucagon and lipogenic precursors *in vitro* is dependent on the time of day at which the hepatocytes were prepared (Gibbons *et al.*, 1984). Similar diurnal variations in the sensitivity of cholesterologenesis to lipogenic precursors and inhibitors have also been observed (Björnsson *et al.*, 1985; Gibbons *et al.*, 1986). A third objective of the present work was to determine what effect, if any, fat-feeding has on these diurnal fluctuations and also whether the sensitivity of lipogenesis to pancreatic hormones and lipogenic precursors changes over the 24 h cycle in hepatocytes derived from animals fed on a diet supplemented with unsaturated fat.

EXPERIMENTAL

Maintenance of animals, preparation of hepatocytes and incubation conditions

Groups of male Wistar rats were housed in a windowless room lit artificially between 08:00 h and 20:00 h (Schedule A) or between 16:00 h and 04:00 h (Schedule B). The animals were fed on a commercially available pelleted diet (Diet PRM; E. Dixon and Sons, Ware, Herts., U.K.). This contained (w/w) 47.5% carbohydrate (mainly starch), 2.7% fat and 18.7% protein. Food and water were available *ad libitum*. Other

Abbreviation used: HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA.

groups of rats were maintained under identical conditions but were fed on the above diet which had been supplemented with corn oil (15%, w/w). This diet was prepared by addition of the corn oil to the powdered diet, thoroughly mixing and then reconstituting the pelleted form after addition of water. The pellets were dried at 40 °C overnight. The rats were fed on this diet for 7 days before preparation of the hepatocytes. During this period each rat consumed an average 28.2 ± 0.7 g ($n = 21$) of food per day, and the average weight gain was 35.4 ± 1.2 g ($n = 12$). The final weight of the animals was 262.6 ± 8.7 g ($n = 12$). For animals maintained on the low-fat diet, the corresponding values were: food consumption per day, 25.2 ± 1.0 g ($n = 12$; average weight gain per week 41.7 ± 7.6 g ($n = 12$), final weight 270.0 ± 6.9 g ($n = 12$). There were no significant differences in food consumption, weight gain or final weight between animals in each group.

Hepatocytes were prepared at 10:00 h from animals maintained on each type of diet. For animals on Schedule A, this point was 2 h into the light phase of the cycle (L_2 -hepatocytes); for those on Schedule B, this represented a time 6 h into the dark phase of the cycle (D_6 -hepatocytes). Procedures for the preparation and incubation of hepatocytes have been described previously (Seglen, 1976; Pullinger & Gibbons, 1983, 1985). L-[U- 14 C]Lactate (160 d.p.m./nmol; to give an initial concentration of 2 mM) was added to the hepatocyte suspensions as a solution in incubation medium. Substrate depletion during the incubation was minimized by adding similar quantities after 1 h. A mixture of non-radioactive lactate and pyruvate (to give initial concentrations of 10 mM and 1 mM respectively) was also added as a solution in incubation medium. Glucagon and insulin were added as solutions in 0.9% NaCl to give initial concentrations of 0.1 μ M and 1 m-unit/ml respectively. To compensate for hormone biodegradation (Beynen *et al.*, 1979; McGarry *et al.*, 1978), similar quantities of each hormone were added at 1 h intervals.

Measurement of incorporation of labelled substrates into cholesterol and fatty acids: determination of HMG-CoA reductase activity

In most incubations, the rates of cholesterol and fatty acid synthesis from all carbon sources were determined by addition of $^3\text{H}_2\text{O}$ to the incubation medium to give a final specific radioactivity of 1 mCi/ml. In other incubations, lipid synthesis from L-[U- 14 C]lactate alone was determined. After incubation for 2 h or 3 h at 37 °C, the cell suspension was chilled on ice and the cell pellet was sedimented by centrifugation at 80 g for 1.5 min. The pellet was washed by resuspension in 1.0 ml of Krebs-Henseleit (1932) buffer, followed by re-centrifugation as before. The fatty acid and cholesterol fractions of the cell were isolated as described previously (Gibbons *et al.*, 1986) and their ^3H and ^{14}C radioactivities were determined by scintillation counting.

HMG-CoA reductase activity was determined on samples (0.3 ml) of cell suspensions which were removed at 1 h, 2 h and 3 h after the start of the incubation. The cells were sedimented by centrifugation (2 °C) at 80 g for 1.5 min. The cell pellet was washed by resuspension in Krebs-Henseleit buffer, followed by re-centrifugation under the same conditions. HMG-CoA reductase activity was measured by the method described by

Kandutsch and colleagues (Saucier & Kandutsch, 1979; Cavenee *et al.*, 1981).

Calculation of the absolute rate of cholesterol synthesis and the contribution of L-[U- 14 C]lactate

The weight (nmol) of $^3\text{H}_2\text{O}$ incorporated into cholesterol was determined from its specific radioactivity. The relationship between ^3H and carbon incorporated was determined as described previously (Pullinger & Gibbons, 1983), and this was used to calculate the weight of cholesterol synthesized. The weight (nmol) of L-[U- 14 C]lactate incorporated into cholesterol was calculated from the specific radioactivity of this precursor.

Other analytical methods

Protein was determined by the method of Gieger & Bessman (1972). For determination of plasma non-esterified fatty acids, a sample of blood 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).

Materials

The sources of materials used in this work have been described previously (Gibbons & Pullinger, 1979; Gibbons *et al.*, 1986). In addition, all radiochemicals were obtained from Amersham International, Little Chalfont, Bucks., U.K. Corn oil was purchased locally, and its composition (w/w) was probably similar to that reported previously (i.e. 50% linolenic acid, 34% oleic acid and 13% palmitic acid) (Ide *et al.*, 1978).

RESULTS

Diurnal variations in the effects of an unsaturated-fat-containing diet on hepatic lipogenesis

To determine whether there were any differential effects of the corn-oil-supplemented diet on lipogenesis at different times of the 24 h cycle, hepatocytes from normal or fat-fed animals were prepared either at the mid-point of the dark phase of the cycle (D_6 -hepatocytes) or at a point 2 h into the light phase (L_2 -hepatocytes). These points represent the times of maximum and minimum food intake, respectively, in rats fed *ad libitum* (Fukuda *et al.*, 1985). Each type of hepatocyte preparation was incubated in the standard medium containing $^3\text{H}_2\text{O}$ for 2 h. Previous studies have shown that the rates of lipogenesis in hepatocytes incubated under a variety of conditions remained linear with time during this period (Gibbons *et al.*, 1984). Table 1 shows that, although the fat-supplemented diet gave rise to a significant decrease in lipogenesis of about 38% ($P < 0.05$) in the L_2 -hepatocytes, this effect was more pronounced at D_6 , in which case a 67% increase was observed ($P < 0.05$). These results show changes in the rate of flux of carbon from all endogenous sources into hepatocyte fatty acids. Since lactate is an important precursor of fatty acids in the livers of rats fed on a high-carbohydrate diet (Salmon *et al.*, 1974; Hems *et al.*, 1975; Boyd *et al.*, 1981; Gibbons *et al.*, 1984), it was decided to examine the effects of the high-fat diet on the utilization, specifically, of lactate for lipogenesis. Thus a similar series of experiments were carried out in which [^{14}C]lactate (2 mM)

Table 1. Diurnal variations in the effects of a high-unsaturated-fat diet on lipogenesis in hepatocytes

Hepatocyte suspensions (3.0 ml) were incubated for 2 h in the presence of either $^3\text{H}_2\text{O}$ (1 mCi/ml) or [^{14}C]lactate (2 mM; 160 d.p.m./nmol). After 2 h, the incorporation of ^3H or ^{14}C into the fatty acid fraction of the cells was determined. Plasma non-esterified fatty acid (NEFA) concentration was determined on blood taken from the descending vena cava. Numbers in parentheses represent the numbers of independent hepatocyte preparations in each group. Percentage values in parentheses refer to the effects of the high-fat diet expressed as a percentage of the respective control (chow only) values.

Time of day	Type of diet					
	Chow only		Chow plus unsaturated fat			
	Fatty acids synthesized (nmol of ³ H ₂ O/mg of protein)	Lactate converted into fatty acids (nmol/mg of protein)	Plasma NEFA concn. (mM)	Fatty acids synthesized (nmol of ³ H ₂ O/mg of protein)	Lactate converted into fatty acids (nmol/mg of protein)	Plasma NEFA concn. (mM)
L ₂	54.7 ± 8.2 (8)	21.7 ± 2.8 (8)	0.34 ± 0.03	33.6 ± 4.8 (6) (61.4%)	6.6 ± 1.6 (6) (30.4%)	0.49 ± 0.04 (144.1%)
D ₆	31.5 ± 8.5 (12)	7.4 ± 3.2 (8)	0.34 ± 0.04	10.6 ± 1.4 (6) (33.6%)	1.24 ± 0.26 (6) (16.7%)	0.47 ± 0.04 (138.2%)

was added to each type of hepatocyte suspension instead of $^3\text{H}_2\text{O}$ (Table 1). In this case, in both the D_6 - and L_2 -hepatocytes, the inhibitory effect of the high-fat diet on lipogenesis from lactate was far more pronounced than that on lipogenesis from all carbon sources. Nevertheless, as was the case when $^3\text{H}_2\text{O}$ was used, lipogenesis from [^{14}C]lactate at D_6 was far more sensitive to inhibition than that at L_2 .

Diurnal variations in the effects of an unsaturated-fat-containing diet on hepatic cholesterol synthesis

Similar series of experiments were carried out to determine whether the above dietary changes had any differential effects on the rate of cholesterol synthesis, and on HMG-CoA reductase activity in hepatocytes prepared at different times of the day. In this case, hepatocytes were incubated under conditions identical with those described above and the rates of $^3\text{H}_2\text{O}$ incorporation into cholesterol were determined. These values were employed to calculate carbon flux from all endogenous sources into cholesterol (see the Experimental section). The specific utilization of [^{14}C]lactate for cholesterol synthesis was also determined in each type of hepatocyte preparation. These results are shown in Table 2. In hepatocytes prepared from normal animals at L_2 , the rate of cholesterol synthesis was lower than in those prepared at D_6 ($P < 0.001$), in agreement with previous observations from this laboratory (Björnsson *et al.*, 1985). However, although at L_2 the corn-oil-supplemented diet had no significant inhibitory effect, at D_6 the rate of cholesterol synthesis was suppressed to a highly significant extent ($P < 0.001$). This differential effect of fat-feeding eliminated the normal diurnal variation of cholesterol synthesis. The unsaturated-fat diet had similar, but more pronounced, effects on carbon flux from lactate into cholesterol. In this case, although an inhibitory effect was observed in the L_2 -hepatocytes, this was not significant. However, there was a marked significant inhibition in the hepatocytes prepared at D_6 ($P < 0.01$).

Similar differential effects of the high-unsaturated-fat diet were also observed on the activity of HMG-CoA reductase measured immediately in hepatocytes prepared at D_6 and L_2 (i.e. at zero time in Fig. 1). On incubation, irrespective of the time of day at which the hepatocytes were prepared from the fat-fed animals, HMG-CoA reductase activity remained constant or declined somewhat over a 2 h period. This was in sharp contrast with the pattern of change observed on incubation of hepatocytes from the normal animals (Fig. 1).

Effects of insulin, glucagon and lactate on lipogenesis in hepatocytes from animals fed on a high-unsaturated-fat diet

The stimulatory and inhibitory effects, respectively, of insulin and glucagon on hepatic lipogenesis in animals fed on a high-carbohydrate diet are well established (Geelen *et al.*, 1980). The question as to whether these effects are modified in the livers of animals fed on fat-supplemented diets has not been systematically investigated. To address this problem, hepatocytes from normal and fat-fed animals were incubated for 3 h in the presence or absence of insulin (1 m-unit/ml) or glucagon (0.1 μM). Previous studies have shown that these hormone concentrations were maximally effective in modifying the rates of lipogenesis in hepatocytes from

Table 2. Diurnal variations in the effects of a high-unsaturated-fat diet on cholesterol synthesis in hepatocytes

Hepatocyte suspensions (3.0 ml) were incubated in the presence of $^3\text{H}_2\text{O}$ (1 mCi/ml) or [^{14}C]lactate (2 mM; 160 d.p.m./nmol) for 2 h. The incorporation of ^3H or ^{14}C into cellular cholesterol was then determined. The weight of cholesterol synthesized was determined as described in the Experimental section. The numbers in parentheses represent the numbers of independent hepatocyte preparations in each group. The percentage values in parentheses refer to the effects of the unsaturated-fat diet compared with the respective control (chow only) values.

Time of day	Type of diet			
	Chow only		Chow plus unsaturated fat	
	Cholesterol synthesized (nmol/mg of protein)	Lactate converted into cholesterol (nmol/mg of protein)	Cholesterol synthesized (nmol/mg of protein)	Lactate converted into cholesterol (nmol/mg of protein)
L_2	1.71 ± 0.05 (8)	5.85 ± 1.35 (7)	1.65 ± 0.29 (6) (96.5%)	3.24 ± 0.63 (6) (55.4%)
D_6	3.01 ± 0.03 (9)	6.75 ± 1.35 (7)	1.15 ± 0.04 (6) (38.2%)	1.98 ± 0.09 (6) (29.3%)

Fig. 1 Changes in the activity of HMG-CoA reductase during incubation of hepatocytes from rats fed on a normal or a high-unsaturated-fat-containing diet

Hepatocyte suspensions (3.0 ml) were incubated in Krebs–Henseleit (1932) buffer containing albumin as described in the Experimental section. HMG-CoA reductase activity was measured in a sample of cells (0.3 ml) before incubation and in similar samples taken at 1, 2 and 3 h. Each point is the average $\pm$ S.E.M. of determinations on hepatocytes from at least six rats. (a) Hepatocytes prepared at D_6 ; (b) hepatocytes prepared at L_2 ; ●, normal hepatocytes; ▲, fat-diet hepatocytes.

Table 3. Effects of insulin and glucagon on the rates of lipogenesis in hepatocytes from normal and unsaturated-fat-fed animals at different times of the day

Hepatocytes were incubated for 3 h in the presence of $^3\text{H}_2\text{O}$ (1 mCi/ml) either alone or with insulin or glucagon. The incorporation of ^3H into the fatty acid fraction of the cells was then determined. The numbers in parentheses refer to the numbers of independent hepatocyte preparations in each group.

Time of day	Additions to hepatocytes	Type of diet			
		Chow only		Chow plus unsaturated fat	
		(nmol of $^3\text{H}_2\text{O}$ /mg of protein)	(% change)	(nmol of $^3\text{H}_2\text{O}$ /mg of protein)	(% change)
L_2	—	77.4 ± 14.4 (6)		43.2 ± 5.3 (4)	
	Insulin (1 munit/ml)	105.4 ± 20.3 (6)	+36.2	66.6 ± 10.9 (4)	+54.2
	Glucagon (0.1 μM)	14.8 ± 1.1 (4)	−80.8	130 ± 1.9 (4)	−70
D_6	—	54.3 ± 16.7 (6)		12.1 ± 2.3 (4)	
	Insulin (1 munit/ml)	72.6 ± 24.2 (6)	+33.7	14.1 ± 2.2 (4)	+16.5
	Glucagon (0.1 μM)	15.5 ± 1.8 (6)	−71.5	7.9 ± 1.2 (4)	−35

Table 4. Effect of lipogenic precursors, with and without insulin, on the rate of lipogenesis in rats fed on a normal or unsaturated-fat-supplemented diet

Hepatocytes prepared at D₆ were incubated in the presence of ³H₂O (1 mCi/ml) for 3 h either alone or in the presence of the additional hormones and substrates indicated below. At the end of this period the incorporation of ³H into the cellular fatty acids was determined.

Addition(s) to flask	Type of diet			
	Chow only		Chow plus unsaturated fat	
	Fatty acids synthesized (nmol of ³ H ₂ O/ mg of protein)	Effect of addition (% of control)	Fatty acids synthesized (nmol of ³ H ₂ O/ mg of protein)	Effect of addition (% of control)
—	74.6 ± 16.9	100	16.4 ± 2.2	100
Lactate + pyruvate	122.7 ± 12.4	164.5	47.1 ± 8.2	287.2
Lactate + pyruvate + insulin	174.3 ± 14.0	233.6	59.6 ± 9.8	363.4

animals fed on a normal diet. The rates of lipogenesis also remain linear with time for 3 h under these conditions (Gibbons *et al.*, 1984). In view of the varying effectiveness of these hormones in modifying the rates of lipogenesis at different times over the diurnal cycle of normal animals (Gibbons *et al.*, 1984) and of the differential effects of the fat-supplemented diet at different times of the day (see above), these experiments were carried out in hepatocytes obtained at both L₂ and D₆. The results of this experiment are given in Table 3. Here again, it was apparent that the high-fat diet was more inhibitory towards lipogenesis at D₆ than at L₂. In addition, although at L₂ insulin stimulated and glucagon inhibited lipogenesis to approximately the same extent in hepatocytes from the normal-fed and fat-fed animals, at D₆ these relative effects were quantitatively dissimilar. In this case, in the hepatocytes from the fat-fed animals, the effect of both these hormones was blunted compared with that observed in the normal hepatocytes (Table 3).

The above results have shown that a diet high in unsaturated fat produced a large decrease in lipogenesis when hepatocytes were prepared mid-way through the dark phase of the 24 h cycle. Lipogenesis is also suppressed in hepatocytes obtained from starved animals, and this low rate may be rapidly enhanced *in vitro* by incubating hepatocytes in a medium containing a high concentration of lipogenic precursors such as lactate and pyruvate (Boyd *et al.*, 1981; Gibbons *et al.*, 1984). In the present work it was decided to determine whether the unsaturated-fat-mediated suppression of hepatic lipogenesis could also be reversed *in vitro* under similar conditions. To investigate this, D₆-hepatocytes obtained from rats maintained on each type of diet were incubated with a mixture of lactate (10 mM) and pyruvate (1 mM). Parallel incubations were carried out in which, in addition to the substrate mixture, insulin (1 m-unit/ml) was also present (Table 4). The lactate plus pyruvate substrate mixture was capable of stimulating lipogenesis in fat-fed animals about 3-fold, a factor which was considerably greater than that observed in comparable incubations with hepatocytes from rats fed on the normal diet. Despite this, however, the maximum lipogenic capacity of the hepatocytes, measured, in each case, in

the presence of lactate plus pyruvate, remained lower in the fat-fed rats than in those fed on a normal diet. Further supplementation of the medium with insulin led to an additional, although much smaller, stimulation of lipogenesis compared with that observed with the substrate mixture. This additional effect of insulin was smaller (26.5% stimulation) in rats fed on the unsaturated-fat-supplemented diet compared with that in the animals receiving the normal diet (42.1%). These results are thus consistent with the lower sensitivity to insulin of basal lipogenesis in animals fed on the higher-fat diet (Table 3).

DISCUSSION

Importance of the diurnal cycle in determining the response of lipid synthesis to a higher-fat diet

All the differences in hepatic lipid metabolism resulting from inclusion of unsaturated fat in the diet were much more pronounced in hepatocytes prepared mid-way through the dark phase of the cycle. These differences included the extent of the inhibitory effect on lipogenesis, cholesterol synthesis and initial HMG-CoA reductase activity, and the changes in the sensitivity of lipogenesis to stimulation by insulin and inhibition by glucagon. In most cases, the effects observed at D₆ were not apparent at L₂ and were thus limited to the relatively short period of time during which the animals consume most of their food (Fukuda *et al.*, 1985). After this time, the characteristics of hepatic lipid metabolism rapidly reverted to those of animals consuming a normal diet. The overall effect of this differential response to fat-feeding on the diurnal patterns of hepatic lipid metabolism was an accentuation of the amplitude of the rhythm for lipogenesis and a dampening of that for cholesterol synthesis. It is of course possible that these effects of unsaturated fat, which in the present work was included in the diet for 7 days, may become modified after feeding for a longer period. Nevertheless, the present differences in the effects of an unsaturated-fat diet emphasize the importance of the strict timing of experiments in interpreting the effects of dietary changes in hepatic lipid metabolism.

Mechanism of the hepatic response to unsaturated-fat feeding

Consumption of a diet high in fat leads to an increase in the concentration of plasma non-esterified fatty acids. The resulting increase in the concentration of hepatic fatty acyl-CoA is thought to be one of the factors which contribute to a decreased activity of the lipogenic enzymes pyruvate dehydrogenase (EC 1.2.2.2; Rosmos & Leveille, 1974; Denton & Hughes, 1978) and acetyl-CoA carboxylase (EC 6.4.1.2; Rosmos & Leveille, 1974; Kim, 1983). In the present work, although the unsaturated-fat diet produced similar increases in plasma non-esterified-fatty-acid concentrations at both L_2 ($P < 0.02$) and D_6 ($P < 0.05$) (Table 1), the decrease in the rate of lipogenesis was much greater during the latter period. This result indicates, perhaps, that these effects are due to factors other than, or in addition to, changes in plasma non-esterified fatty acids.

The inhibitory effect of the high-fat diet on the conversion of lactate into fatty acids was greater than that on the overall rate of fatty acid synthesis from all sources (Table 1). This suggests, firstly, that pyruvate dehydrogenase was more sensitive to inhibition than the other regulatory enzymes of lipogenesis and, second, that during fat-feeding sources of carbon other than those derived from carbohydrate oxidation become relatively more important for lipogenesis. Despite this inhibitory effect of fat-feeding on lactate utilization, a higher concentration of lactate was able to stimulate carbon flux from all sources into fatty acids (Table 4). This effect may be related to the stimulation of pyruvate dehydrogenase by pyruvate in liver mitochondria from starved rats (Portenhauser & Wieland, 1972). Alternatively, or in addition, lactate has been reported to increase the utilization of carbon, from other sources, for lipogenesis (Salmon *et al.*, 1974). In the present work, this effect may contribute to the increase in the rate of lipogenesis in the presence of the lactate/pyruvate mixture in hepatocytes from the fat-fed animals. This partial reversal of the dietary-induced decrease in hepatic lipogenesis by lactate and pyruvate *in vitro* was similar to that observed when hepatocytes from starved animals were incubated under similar conditions (Boyd *et al.*, 1981; Gibbons *et al.*, 1984). This latter information suggested strongly that in starved animals one of the major factors limiting the rate of lipogenesis was lack of substrate generated extrahepatically and normally supplied to the liver as lactate. If this is the case, then, in the present work, it would appear that the extrahepatic production of lactate was inhibited in some way by inclusion of unsaturated fat in the diet. Furthermore, in the present work, even though lipogenic substrates such as lactate and pyruvate were present in non-limiting concentrations, the rate of lipogenesis in hepatocytes from the fat-fed rats remained lower than that in normal hepatocytes incubated under identical conditions (Table 4). It would therefore appear that, in addition to changes in the rate of substrate supply, fat-feeding also decreased the capacity of the lipogenic enzymes. It must be emphasized, however, that this decrease was rapidly reversible and became much less apparent during that part of the cycle when the animals' food intake was low. It should also be noted that, *in vivo*, diurnal changes in the availability of lactate, produced extrahepatically, may be important in contributing to the diurnal variation of hepatic lipogenesis. In the

present work, any such changes were not simulated by varying the concentration of lactate in the incubation medium. This may contribute to the difference in the time at which the maximal rate of hepatic lipogenesis was observed in the present work *in vitro*, and by others *in vivo* (Hems *et al.*, 1975; Fukuda *et al.*, 1985).

The inhibitory effects of the high-fat diet on the rate of hepatocyte cholesterogenesis broadly paralleled those on lipogenesis. For instance, the inhibition was more pronounced at D_6 than at L_2 . Also, at each time point, the relatively greater inhibition of lactate conversion into cholesterol compared with that of cholesterogenesis from all-sources suggested that, during fat-feeding, sources of carbon other than those derived from carbohydrate oxidation became relatively more important for cholesterogenesis, as well as for lipogenesis. The greater decrease in HMG-CoA reductase activity in freshly prepared hepatocytes at D_6 after fat-feeding, compared with that at L_2 , was also consistent with the relative effect of this dietary change in cholesterogenesis at the two time points in question. Fatty acyl-CoA derivatives inhibit microsomal HMG-CoA reductase (Lehrer *et al.*, 1981), and it is possible that the increased plasma non-esterified fatty acids at D_6 contributed to the inhibitory effects on HMG-CoA reductase and cholesterogenesis at this time. If this was the case, then, again, it is difficult to explain why similarly high concentrations of plasma non-esterified fatty acid at L_2 had such little effect on these parameters.

Decreased responsiveness of hepatocyte lipogenesis to insulin and glucagon

It has been reported previously that the relative extent of stimulation by insulin, and inhibition by glucagon, of hepatocyte lipogenesis was dependent on the time of day at which the cells were prepared (Gibbons *et al.*, 1984). The present results provide evidence that the responsiveness of lipogenesis to these hormones is also influenced by the unsaturated-fat content of the diet (Table 3). Furthermore, the extent to which fat-feeding modified the lipogenic response to insulin and glucagon was also dependent on the time of day at which the hepatocytes were prepared. In this respect the response was blunted at D_6 , but very little difference in the hormonal effect was observed at L_2 . As regards insulin, the decreased responsiveness of lipogenesis at D_6 in the fat-fed animals may have arisen from insulin resistance, which is induced in both liver (Sun *et al.*, 1977) and adipose tissue (Ip *et al.*, 1976; Hissin *et al.*, 1982) as a result of a decrease in the number of insulin receptors. If this is the case, then the present results indicate that insulin resistance varied with a diurnal periodicity and that the liver rapidly re-acquired normal insulin sensitivity during that period over which food (and thus dietary fat) intake was low.

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REFERENCES

- Abraham, S., Hillyard, L. A., Lin, C. Y. & Schwartz, R. S. (1983) *Lipids* **18**, 820–829
- Baltzell, J. K. & Berdanier, C. D. (1985) *J. Nutr.* **115**, 104–110
- Beynen, A. C., Vaartjes, W. J. & Geelen, M. J. H. (1979) *Diabetes* **28**, 828–835
- Björnsson, O. G., Pullinger, C. R. & Gibbons, G. F. (1985) *Arch. Biochem. Biophys.* **238**, 135–145

- Boyd, M. E., Albright, E. B., Foster, D. W. & McGarry, J. D. (1981) *J. Clin. Invest.* **68**, 142–152
- Cavenee, W. K., Chen, H. W. & Kandutsch, A. A. (1981) *J. Biol. Chem.* **256**, 2675–2681
- Denton, R. M. & Hughes, W. A. (1978) *Int. J. Biochem.* **9**, 545–552
- Edwards, P. A., Muroya, H. & Gould, R. G. (1972) *J. Lipid Res.* **13**, 396–401
- Fukuda, H., Katsurada, A. & Iritani, N. (1985) *Biochim. Biophys. Acta* **835**, 163–168
- Geelen, M. J. H., Harris, R. A., Beynen, A. C. & McCune, S. A. (1980) *Diabetes* **29**, 1006–1022
- Gibbons, G. F. & Pullinger, C. R. (1979) *Biochem. J.* **177**, 255–263
- Gibbons, G. F., Pullinger, C. R., Munday, M. R. & Williamson, D. H. (1983) *Biochem. J.* **212**, 843–848
- Gibbons, G. F., Pullinger, C. R. & Björnsson, O. G. (1984) *J. Lipid Res.* **25**, 1358–1367
- Gibbons, G. F., Atwell Thomas, C. P. & Pullinger, C. R. (1986) *Biochem. J.* **235**, 19–24
- Gieger, P. J. & Bessman, S. P. (1972) *Anal. Biochem.* **49**, 467–473
- Hems, D. A., Rath, E. A. & Verrinder, T. R. (1975) *Biochem. J.* **150**, 167–173
- Hissin, P. J., Karnieli, E., Simpson, I. A., Salans, L. B. & Cushman, S. W. (1982) *Diabetes* **31**, 589–592
- Ide, T., Okamatsu, H. & Sugano, M. (1978) *J. Nutr.* **108**, 601–612
- Ip, C., Tepperman, H. M., Halohan, P. & Tepperman, J. (1976) *J. Lipid Res.* **17**, 588–599
- Jenke, H.-S., Löwel, M. & Berndt, J. (1982) *Hoppe-Seyler's Z. Physiol. Chem.* **363**, 725–730
- Kim, K.-H. (1983) *Curr. Top. Cell. Regul.* **22**, 143–176
- Krebs, H. A. & Henseleit, K. (1932) *Hoppe-Seyler's Z. Physiol. Chem.* **210**, 33–66
- Lehrer, G., Panini, S. R., Rogers, D. H. & Rudney, H. (1981) *J. Biol. Chem.* **256**, 5612–5619
- McGarry, J. D., Takabayashi, Y. & Foster, D. W. (1978) *J. Biol. Chem.* **253**, 8294–8300
- Munday, M. R. & Williamson, D. H. (1983) *Biochem. J.* **214**, 183–187
- Portenhauser, R. & Wieland, O. (1972) *Eur. J. Biochem.* **31**, 308–314
- Pullinger, C. R. & Gibbons, G. F. (1983) *J. Lipid Res.* **24**, 1321–1328
- Pullinger, C. R. & Gibbons, G. F. (1985) *Biochim. Biophys. Acta* **833**, 44–51
- Rodwell, V. W., Norstrom, J. L. & Mitschelen, J. J. (1976) *Adv. Lipid Res.* **14**, 1–74
- Rosmos, D. R. & Leveille, G. A. (1974) *Adv. Lipid Res.* **12**, 97–146
- Salmon, D. M. W., Bowen, N. L. & Hems, D. A. (1974) *Biochem. J.* **142**, 611–618
- Saucier, S. E. & Kandutsch, A. A. (1979) *Biochim. Biophys. Acta* **572**, 541–556
- Scott, D. F. & Potter, V. R. (1970) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **29**, 1553–1559
- Seglen, P. O. (1976) *Methods Cell Biol.* **13**, 29–83
- Shimizu, S., Inoue, K., Tani, Y. & Yamada, H. (1979) *Anal. Biochem.* **98**, 341–345
- Sun, J. V., Tepperman, H. M. & Tepperman, J. (1977) *J. Lipid Res.* **18**, 533–539
- Triscari, J., Hamilton, J. G. & Sullivan, A. C. (1978) *J. Nutr.* **108**, 815–825

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