The activities of lipoprotein lipase and of enzymes involved in triacylglycerol synthesis in rat adipose tissue

Effects of starvation, dietary modification and of corticotropin injection

Nigel LAWSON,* Anne D. POLLARD,* Richard J. JENNINGS,* Michael I. GURR†‡ and David N. BRINDLEY*

**Department of Biochemistry, University of Nottingham Medical School, Queen's Medical Centre, Nottingham NG7 2UH, and †Nutrition and Agriculture Division, Unilever Research Laboratory, Colworth House, Sharnbrook, Bedford MK44 1LQ, U.K.*

(Received 14 May 1981/Accepted 21 August 1981)

1. The effects of dietary modification, including starvation, and of corticotropin injection on the activities of acyl-CoA synthetase, glycerol phosphate acyltransferase, dihydroxyacetone phosphate acyltransferase, phosphatidate phosphohydrolase, diacylglycerol acyltransferase and lipoprotein lipase were measured in adipose tissue. 2. Lipoprotein lipase activities in heart were increased and those in adipose tissue were decreased when rats were fed on diets enriched with corn oil or beef tallow rather than with sucrose or starch. The lipoprotein lipase activity was lower in the adipose tissue of rats fed on the sucrose rather than on the starch diet. 3. Rats fed on the beef tallow diet had slightly higher activities of the total glycerol phosphate acyltransferase in adipose tissue than did rats fed on the sucrose or starch diet. The diacylglycerol acyltransferase and the mitochondrial glycerol phosphate acyltransferase activities were higher for the rats fed on the tallow diet than for those fed on the corn-oil diet. 4. Starvation significantly decreased the activities of lipoprotein lipase (after 24 and 48 h), acyl-CoA synthetase (after 24 h) and of the mitochondrial glycerol phosphate acyltransferase and the *N*-ethylmaleimide-insensitive dihydroxyacetone phosphate acyltransferase (after 48 h) in adipose tissue. The activities of the microsomal glycerol phosphate acyltransferase, diacylglycerol acyltransferase and the soluble phosphatidate phosphohydrolase were not significantly changed after 24 or 48 h of starvation. 5. The activities of lipoprotein lipase and phosphatidate phosphohydrolase in adipose tissue were decreased 15 min after corticotropin was injected into rats during November to December. No statistically significant differences were found when these experiments were performed during March to September. These differences may be related to the seasonal variation in acute lipolytic responses. 6. These results are discussed in relation to the control of triacylglycerol synthesis and lipoprotein metabolism.

The present paper investigates the activities of the enzymes that might be involved in controlling the uptake and esterification of fatty acids by adipose tissue. Emphasis is placed on the comparisons of this

Abbreviations used: DHAPAT, dihydroxyacetone phosphate acyltransferase (EC 2.3.1.42); GPAT, glycerol phosphate acyltransferase (EC 2.3.1.15); LPL, lipoprotein lipase (EC 3.1.1.34); PAP, phosphatidate phosphohydrolase (EC 3.1.3.4).

‡ Present address: National Institute for Research in Dairying, Shinfield, Reading, U.K.

control with that which operates in the liver. It is expected that the rate of triacylglycerol synthesis will be high in both of these organs when animals consume large quantities of fat or carbohydrate. Conversely, in starvation and in stress conditions, triacylglycerol is broken down in adipose tissue and the fatty acids are transported to other organs including the liver. It is reasonable to assume that the activation of lipolysis in adipose tissue under these conditions may be accompanied by a simultaneous decrease in the uptake and esterification of fatty acids. It is thought that LPL controls the first

part of this process and that its activity is mainly promoted by insulin (Ashby *et al.*, 1979). There is also evidence that the activities of the enzymes involved in triacylglycerol synthesis in adipose tissue are decreased if the availability of catecholamines relative to insulin increases (Saggerson *et al.*, 1979). However, there is relatively little evidence at present to confirm that the enzymes involved in esterification are controlled *in vivo*.

We decided to investigate whether this evidence could be provided by measuring these activities in rats that had been (a) starved; (b) maintained on different diets, (c) fed acutely with various nutrients or (d) injected with corticotropin. Furthermore the activity of LPL was determined in these experiments to compare the magnitude of its change relative to those of the enzymes involved in esterification. Particular attention was paid to the ability to metabolize phosphatidate, since this is an important site of control in the liver (Brindley *et al.*, 1979a; Lehtonen *et al.*, 1979; Brindley, 1981). Its effective activity in liver might also be modified by the action of the phosphatidate deacylase system that could compete for substrate (Sturton *et al.*, 1978; Sturton & Brindley, 1980). However, it is not yet known whether this deacylase system exists in adipose tissue, and if so whether it might have a regulatory function.

Materials and methods

Materials and animals

The sources of these materials and the treatment of the rats has been described previously (Sturton & Brindley, 1978; Lawson *et al.*, 1981). All radiochemicals were from The Radiochemical Centre, Amersham, Bucks., U.K.

Preparation of cell fractions

The microsomal and soluble fractions of rat epididymal fat-pads were prepared essentially as described by McKeel & Jarrett (1970). These fractions were then dialysed overnight (about 16 h) at 4°C against two changes of 100 vol. of 0.25 M-sucrose (adjusted to pH 7.4 with KHCO_3) containing 0.5 mM-dithiothreitol. This procedure removes P_i and bivalent cations.

Enzyme activities

The mean activity of each sample was calculated from initial rates obtained at three different protein concentrations. These were expressed per mg of protein, except for LPL activity, which was calculated per g of tissue, since acetone powders were used for this assay.

Acyl-CoA synthetase (EC 6.2.1.3)

This was assayed in an incubation volume of 0.25 ml containing 75 mM-Tris, adjusted to pH 7.4 with HCl, 4 mM-dithiothreitol, 5 mM-ATP, 30 mM- MgCl_2 , 200 μM -CoA, 1.6 mM-potassium [9,10- ^3H]-palmitate (sp. radioactivity 1 Ci/mol), 6 mg of fatty-acid-poor bovine serum albumin/ml and up to 80 μg of homogenate protein. Incubations were for 5 min at 37°C. They were stopped by the addition of Dole reagent and the concentration of palmitoyl-CoA was then determined (Lloyd-Davies & Brindley, 1975).

Determination of total and mitochondrial GPAT activities

Each assay contained, in a final volume of 0.25 ml, 25 mM-Tris adjusted to pH 7.4 with HCl, 50 mM-KCl, 100 μM -palmitoyl-CoA, 1 mM-*sn*-[1,3- ^3H]glycerol 3-phosphate (sp. radioactivity 1 Ci/mol), 7.1 mg of fatty-acid-poor bovine serum albumin/ml and 56 μg of homogenate protein. Reactions were for 10 min at 37°C and they were then stopped with 1 ml of butan-1-ol that had been equilibrated with 0.1 M-HCl containing 10 mM-*rac*-glycerol 3-phosphate. A solution (750 μl) of 0.1 M-HCl containing 10 mM-*rac*-glycerol 3-phosphate that had been saturated with butan-1-ol was added and the phases were separated by centrifugation. The butanol phase was washed twice with 600 μl portions of the aqueous solution described above and the ^3H in the butanol phase was determined by liquid-scintillation counting. This gives the total GPAT activity. Portions of the homogenate were also incubated for 10 min at 4°C with 10 mM-*N*-ethylmaleimide (Saggerson *et al.*, 1980). Excess *N*-ethylmaleimide was removed by adding a final concentration of 20 mM-dithiothreitol. This preparation was then assayed for GPAT activity as described above, but by using up to 250 μg of protein. The *N*-ethylmaleimide-insensitive activity was assumed to be equivalent to the mitochondrial GPAT (Saggerson *et al.*, 1980). It was subsequently found that the addition of 2 mM- CaCl_2 and 3 mM- CaCl_2 respectively increased the mitochondrial and total GPAT activities by 1.25- and 2-fold respectively. These additions were made for the experiments described in Table 2.

Determination of DHAPAT activity

Samples of the homogenate were treated with 5 mM-*N*-ethylmaleimide (Saggerson *et al.*, 1980; Lawson *et al.*, 1981) to inhibit the microsomal GPAT that can also use dihydroxyacetone phosphate as a substrate (Schlossman & Bell, 1976). The assay system was that used by Lawson *et al.* (1981), except that the pre-incubation volume was 120 μl and it contained 0.2 mM-[U- ^{14}C]fructose 1,6-bisphosphate (sp. radioactivity 3 Ci/mol). The reaction

was started with up to 180 μ g of homogenate protein in a volume of 130 μ l.

Measurement of phosphatidate breakdown and PAP activity

Unless stated to the contrary each assay contained, in 0.25 ml, 100 mM-Tris buffer, adjusted to pH 7.4 with HCl, 0.5 mg of fatty-acid-poor bovine serum albumin, 1 mM-dithiothreitol, 0.2 mM-EGTA, 100 μ g of soluble protein or 75 μ g of microsomal protein, and either 2.5 mM-MgCl₂ or 5 mM-MgCl₂ with microsomal and soluble fractions respectively. The assays for the microsomal fraction were started with a mixed emulsion of 0.65 mM-phosphatidylcholine and 1.5 mM-1,2-diacyl-*sn*-[³H]glycerol 3-phosphate (K⁺ salt) that had previously been sonicated together, and were terminated after 15 min at 37°C. The soluble-fraction assays were treated similarly except that 1 mM-phosphatidylcholine was used instead of 0.65 mM. The assays were sometimes performed in parallel and were then terminated: (a) with 0.61 M-trichloroacetic acid to measure P_i (Bowley *et al.*, 1977); (b) with 1.88 ml of chloroform/methanol (1:2, v/v), followed by the separation of water-soluble and lipid fractions after adding 0.625 ml of chloroform and 0.875 ml of 0.1 M-HCl; or (c) by adding 0.5 ml of butan-1-ol (Bjerve *et al.*, 1974) so that lysophosphatidate and water-soluble products could be separated. The radioactivity in lipids and water-soluble products was identified by t.l.c. on plates of silica-gel G and microgranular cellulose respectively (Sturton & Brindley, 1978).

In the experiments described in Tables 1 and 2 and in Fig. 2, PAP activities were determined by measuring the rate of P_i production. The soluble PAP was determined by using the assay system described above. Mg²⁺-dependent and Mg²⁺-independent activities in the homogenates were measured in similar assay systems, except that either 2.5 mM-MgCl₂ or 1 mM-EDTA was added respectively and no phosphatidylcholine was present.

*Measurement of the degradation of 1,2-diacyl-*sn*-[³H]glycerol and *sn*-[³H]glycerol 3-phosphate*

The assay systems were essentially similar to those described for the degradation of phosphatidate. The degradation of 60 nmol of 1,2-diacyl-*sn*-[³H]glycerol (sp. radioactivity 1 Ci/mol) was measured by adding it as a mixed emulsion with 360 nmol of unlabelled phosphatidate. The reaction was stopped with chloroform/methanol and the products were analysed as described above. Alternatively 60 nmol of *sn*-[1,3-³H]glycerol 3-phosphate (sp. radioactivity 1 Ci/mol) was added and incubated with 360 nmol of phosphatidate. The reactions were stopped with 0.5 ml of butan-1-ol and the products were analysed as described above.

Determination of diacylglycerol acyltransferase activity (EC 2.3.1.20) and ethanol acyltransferase activity

Assays were performed in stoppered tubes by using a method based on that of Coleman & Bell (1976). Each tube contained in a final volume of 200 μ l: 175 mM-Tris, adjusted to pH 8 with HCl, 8 mM-MgCl₂, 30 μ M-[9,10-³H]palmitoyl-CoA (sp. radioactivity 100 Ci/mol) and 1 mg of fatty-acid-poor bovine serum albumin/ml. Reactions were started with 20 μ l of 1.25 mM-*sn*-1,2-diacylglycerol in ethanol or 20 μ l of ethanol alone, followed immediately by the homogenate protein (10–30 μ g). The final concentrations of diacylglycerol and ethanol were 125 μ M and 10% (v/v) respectively. Reactions were stopped after 20 min at 23°C by adding 1.5 ml of propan-2-ol/heptane/water (40:10:1, by vol.) and lipids were extracted (Coleman & Bell, 1976). Triacylglycerols (R_F = 0.25) and ethyl palmitate (R_F = 0.52) were separated on t.l.c. plates of silica-gel 60 (Merck) by development with light petroleum (b.p. 40–60°C)/diethyl ether/acetic acid (90:10:1, by vol.) and the radioactivity in these lipids was determined.

The formation of ethyl palmitate in this type of system was reported by Bakken *et al.* (1979), but the t.l.c. system used by these authors did not enable us to resolve it from triacylglycerol. In our system there was no significant synthesis of triacylglycerol when no diacylglycerol or ethanol were added, or when these were added separately. In the latter case ethyl [³H]palmitate was detected. The rate of ³H incorporation into this compound was decreased by 50 $\pm$ 7% (mean $\pm$ s.d. from three independent experiments) when diacylglycerol was added. This means that the true diacylglycerol acyltransferase activity cannot be obtained by subtracting the rate of [³H]palmitate incorporation into neutral lipid in the presence of ethanol from that in the presence of diacylglycerol plus ethanol. Therefore it was determined by measuring the rate of [³H]palmitate incorporation into the triacylglycerol isolated by t.l.c. Ethanol acyltransferase activity was determined by measuring the incorporation of [³H]palmitate into neutral lipids after the solvent extraction of assays that did not contain diacylglycerol.

Determination of lipoprotein lipase activity

Acetone powders of the total homogenate of the fat-pads and heart were prepared (Salaman & Robinson, 1966) and the activity of lipoprotein lipase was measured by the method of Nilsson-Ehle & Schotz (1976).

Determination of protein concentration and radioactivity

Protein concentrations in Fig. 1 were determined by the method of Tornqvist & Belfrage (1976), and

in Tables 1 and 2 and in Fig. 1 by that of Bradford (1976) except that 0.1% (w/v) Triton X-100 was added to the latter assays to disperse lipid. Radioactive determinations were performed by the method of Manning & Brindley (1972).

Experiments and results

Characterization of the products of phosphatidate degradation in rat adipose tissue

It was important to decide whether phosphatidate deacylase activity is present in adipose tissue and whether this activity changes in different metabolic states. Furthermore, the deacylation of phosphatidate could invalidate the measurement of PAP activity by determining P_i release, since this can also

arise by hydrolysis of glycerol phosphate (Sturton & Brindley, 1978).

Incubation of the microsomal and soluble fractions of rat adipose tissue with phosphatidate leads to the formation of P_i , diacylglycerol and glycerol (Fig. 1). By using the optimum conditions described in the Materials and methods section the total formation of glycerol and diacylglycerol by the microsomal fraction was 10.6 ± 1.9 nmol/min per mg of protein (mean $\pm$ s.d. from four independent experiments) and P_i production was 10.6 ± 2.6 nmol/min per mg of protein. The rates of diacylglycerol plus glycerol synthesis were also equal to P_i production with the soluble fraction; the equivalent values being 13.0 ± 2.2 and 13.7 ± 2.8 . The rates were proportional to the concentration of the cell

Fig. 1. Effects of phosphatidylcholine and Mg^{2+} on the activity of phosphatidate phosphohydrolase from rat adipose tissue. The degradation of phosphatidate by the soluble (a and c) and microsomal (b and d) fractions was assayed by using the optimum conditions described in the Materials and methods section, except that the concentrations of phosphatidylcholine and Mg^{2+} were varied independently as indicated. The formation of glycerol ($\blacktriangle$), diacylglycerol ($\blacksquare$), glycerol plus diacylglycerol ($\blacksquare$) and P_i ($\square$) is shown from a typical experiment.

fraction and were constant with time during the period of incubation.

The addition of phosphatidylcholine as a mixed emulsion with phosphatidate stimulated the formation of P_i by $116 \pm 11\%$ (mean $\pm$ S.D. from four independent experiments) with the soluble fraction. The formation of diacylglycerol and glycerol increased in parallel (Fig. 1a). The addition of 0.65 mM-phosphatidylcholine to incubations containing the microsomal fraction increased the formation of P_i and diacylglycerol by $65 \pm 24\%$ (mean $\pm$ S.D. from four experiments), but it did not significantly alter the production of glycerol (Fig. 1b). Phosphatidylcholine has previously been shown to stimulate the release of P_i from phosphatidate by the soluble fraction of rat adipose tissue (Moller *et al.*, 1977), and by soluble and microsomal fractions of rat liver (Shapiro & Tzur, 1968; Hosaka *et al.*, 1975; Sturton & Brindley, 1978).

The addition of 5 mM- Mg^{2+} to incubations stimulated the rate of P_i release from phosphatidate by $567 \pm 171\%$ (mean $\pm$ S.D. from five independent experiments) and this was accompanied by an increase in the formation of diacylglycerol and glycerol (Fig. 1c). The effect of Mg^{2+} was less pronounced with the microsomal fraction for which 2.5 mM- Mg^{2+} gave stimulations of $74 \pm 18\%$ (mean $\pm$ S.D. from five experiments). Higher concentrations of Mg^{2+} decreased the accumulation of diacylglycerol and the formation of P_i (Fig. 1d). The greater effect of Mg^{2+} on the breakdown of phosphatidate by the soluble fraction is compatible with the conclusions of Jamdar & Fallon (1973), who measured P_i formation.

The addition of Ca^{2+} at concentrations between 0.05–10 mM progressively inhibited the formation of P_i , glycerol and diacylglycerol by both soluble and microsomal fractions. Ca^{2+} (0.5 mM) decreased the formation of glycerol plus diacylglycerol by 25–47% when these fractions were incubated in the absence of Mg^{2+} , or in the presence of optimum concentrations of Mg^{2+} . Control incubations contained 0.2 mM-EGTA, whereas this was omitted when Ca^{2+} was added. These results are compatible with those of Roncari & Hollenberg (1967), who reported that Ca^{2+} inhibited the conversion of phosphatidate into diacylglycerol by fractions from rat adipose tissue.

To be able to interpret the results in Fig. 1 in terms of the activity of phosphatidate phosphohydrolase it is necessary to decide whether any P_i is formed via the deacylation of the phosphatidate to glycerol phosphate and the subsequent hydrolysis of this compound. No evidence for this was obtained since neither lysophosphatidate, nor glycerol phosphate were detected in any incubation. In addition no conversion of *sn*-[3H]glycerol 3-phosphate into [3H]glycerol could be detected during a 30 min incubation with either fraction. It is therefore

likely that the [3H]glycerol was derived from the diacylglycerol that was formed by the action of phosphatidate phosphohydrolase. This possibility is confirmed by the following observation. Incubating 60 nmol of 1,2-diacyl-*sn*-[3H]glycerol with the soluble fraction (100 μ g of protein) and the microsomal fraction (75 μ g of protein) for 30 min resulted in the conversion of 27% and 13% respectively into glycerol. We cannot completely exclude the possibility that some phosphatidate could have been deacylated to lysophosphatidate, which was then converted into monoacylglycerol by phosphatidate phosphohydrolase. However, the deacylation of phosphatidate to glycerol phosphate can be readily demonstrated in liver fractions (Sturton & Brindley, 1978, 1980). Fig. 1 shows that the activity of PAP in rat adipose tissue can be determined by measuring either the formation of P_i , or of diacylglycerol plus glycerol from phosphatidate.

Effects of diet on the activities of various enzymes in adipose tissue and heart

There were no differences in the activities of acyl-CoA synthetase, DHAPAT and the soluble, Mg^{2+} -dependent and Mg^{2+} -independent PAP in adipose tissue (Table 1). The total GPAT activity was slightly greater in rats fed on the tallow diet than in those fed on the starch and sucrose diets. Mitochondrial GPAT and the total DGAT activities were higher for the tallow diet than for rats fed on the corn-oil diet. Ethanol acyltransferase activity was lower in rats fed on the corn-oil diet than for the other three groups. Similar conclusions were drawn when these enzyme activities were expressed per pair of fat-pads, or per g of adipose tissue, since there were only small differences in fat-pad weight and protein content among the dietary groups (Lawson *et al.*, 1981).

LPL activity in adipose tissue was lower in rats fed on the high-fat diets than for the high-carbohydrate diets. It was also lower in rats fed on sucrose rather than on the starch diet. By contrast, this activity in heart was greater in rats fed on the high fat compared with those fed on the high-carbohydrate diets (Table 1).

Effects of corticotropin injection on the activities of LPL and PAP in the adipose tissue of rats fed with various diets

The corticosterone response to the injection of corticotropin was similar in the four groups of rats fed with the experimental diets (Lawson *et al.*, 1981). At 30 min after these injections the circulating concentrations of unesterified fatty acids were 2.1-, 1.8-, 2.0- and 2.0-fold higher ($P < 0.02$ – 0.001) than the basal values for the rats fed on the starch, sucrose, corn-oil and tallow diets respectively (see Table 3 of Lawson *et al.*, 1981). This indicates that lipolysis in adipose tissue was stimulated. There did

Table 1. *Effect of diets on various enzyme activities in adipose tissue and heart*

Male rats were fed for 3 weeks on the diets described by Lawson *et al.* (1981). Enzyme activities that are involved in esterification were determined by using fat-free homogenates, except for the soluble PAP. They are shown as means $\pm$ S.D. for the numbers of rats shown in parentheses. The significance of the differences between groups is shown by: *, $P < 0.05$; **, $P < 0.02$; ***, $P < 0.01$; †, $P < 0.005$; ††, $P < 0.001$.

Tissue	Enzyme activity	Product formed (nmol/min per mg of protein)			
		Starch diet (I)	Sucrose diet (II)	Corn-oil diet (III)	Tallow diet (IV)
Adipose	Acyl-CoA synthetase	71.7 ± 11.9 (8)	81.0 ± 19.9 (8)	61.4 ± 19.7 (8)	76.0 ± 9.8 (8)
	Total GPAT	4.94 ± 1.23 (8)	5.34 ± 0.97 (7)	5.09 ± 1.90 (8)	6.65 ± 1.28 (8)
		I versus IV**	II versus IV*		
	Mitochondrial GPAT	0.53 ± 0.07 (3)	0.46 ± 0.22 (5)	0.48 ± 0.16 (6)	0.64 ± 0.30 (7)
				III versus IV**	
	<i>N</i> -Ethylmaleimide insensitive DHAPAT	0.099 ± 0.087 (7)	0.076 ± 0.031 (8)	0.033 ± 0.056 (7)	0.105 ± 0.037 (7)
	Homogenate PAP (Mg ²⁺ -dependent)	7.74 ± 1.05 (8)	7.65 ± 1.54 (8)	7.48 ± 1.27 (8)	7.78 ± 1.66 (8)
	Homogenate PAP (Mg ²⁺ -independent)	0.95 ± 0.23 (8)	1.05 ± 0.31 (8)	0.89 ± 0.31 (8)	1.25 ± 0.57 (8)
	Soluble PAP	19.9 ± 5.4 (16)	17.1 ± 3.2 (16)	16.9 ± 2.4 (16)	17.1 ± 3.6 (16)
	Diacylglycerol acyltransferase	1.22 ± 0.35 (8)	1.34 ± 0.42 (8)	0.99 ± 0.52 (8)	1.52 ± 0.45 (8)
			III versus IV*		
	Ethanol acyltransferase	0.581 ± 0.160 (8)	0.583 ± 0.107 (8)	0.388 ± 0.085 (8)	0.504 ± 0.079 (8)
		I versus III***	II versus III†	III versus IV**	
		Fatty acid formed (μmol/min per g of tissue)			
Adipose	Lipoprotein lipase	3.06 ± 0.65 (8)	2.39 ± 0.56 (8)	1.45 ± 0.72 (8)	1.51 ± 0.31 (8)
		I versus IV††	I versus II*	I versus III††	II versus IV†
Heart	Lipoprotein lipase	1.34 ± 0.14 (8)	1.56 ± 0.27 (8)	2.07 ± 0.23 (8)	2.17 ± 0.27 (8)
		I versus III††	II versus IV††	II versus III†	I versus IV††

not appear to be any differences in the responses of PAP and LPL amongst the dietary groups, and therefore only examples are shown in Fig. 2. PAP activity remained constant for about 15 min and then decreased by about 20–25% at 30 and 100 min ($P < 0.005$ and 0.01 respectively). It returned to control values by about 200 min and remained relatively constant up to 360 min (Fig. 2). Furthermore, the activity of the soluble PAP was not significantly changed after 360 min (results not shown) when rats were fed with acute loads of fructose, sorbitol, glycerol or ethanol rather than with saline or glucose. The former treatments also increased the concentrations of circulating corticosterone while not altering those of insulin (Brindley *et al.*, 1979b).

The basal activity of LPL in adipose tissue was higher in the rats fed on the high-carbohydrate rather than the high-fat diets (Table 1). The basal activities were retained for about 15 min in all four dietary groups, after which they fell ($P < 0.05$ – 0.005) by about 25–40% between 30 and 360 min after corticotropin injection (Fig. 2).

The results shown in Fig. 2 were obtained during November–December and we repeated these experiments during the same period on two separate occasions with groups of 41B-fed rats. Significant decreases of 25–30% in the activities of PAP ($P < 0.005$) and of 30–35% for LPL ($P < 0.05$) were seen after corticotropin injection and the results in Fig. 2 were confirmed. However, when three separate experiments were performed with groups of 41B-fed rats during March to September there were no statistically significant decreases in the activities of the two enzymes. In one of these experiments there was an apparent increase of 1.4-fold in circulating non-esterified fatty acids, but this was not statistically significant.

Effects of starvation on the activities of various enzymes in adipose tissue

The activity of LPL fell by 62 and 72% respectively after starving rats for 24 and 48 h (Table 2) as was expected from previous work (Robinson, 1970). The activity of acyl-CoA synthetase was decreased by 25% after starvation for 24 h (Table 2), and such a change is compatible with the proposed role of insulin in maintaining this activity (Jason *et al.*, 1976). However, Daniel & Rubenstein (1968) did not find a significant decrease in the activities of acyl-CoA synthetase, total GPAT, soluble PAP and DGAT. Our results for the last three activities agree with this when the results are expressed relative to protein (Table 2), or per pair of fat-pads (results not shown). There were also no significant changes in ethanol acyltransferase activity. Moller *et al.* (1977) found that the soluble PAP activity fell by 38, 48 and 64% after 24, 48 and 72 h of starvation respectively when results were expressed relative to DNA. The decreases relative to protein were 15–22%. Sooranna & Saggerson (1979) have reported a decrease of 19% in the total GPAT and DHAPAT activities of adipocytes after starvation. In the present work starvation for 48 h decreased the activities of the mitochondrial GPAT and the *N*-ethylmaleimide-insensitive DHAPAT by 50% and 58% respectively (Table 2). The differences at 24 h starvation were not statistically significant. Similar conclusions for these enzymes were obtained when the results were expressed per pair of fat-pads or per g of adipose tissue. We are not aware of other results concerning the effects of starvation on these two activities.

It is possible that the enzymes of esterification might be inactivated by a phosphorylation reaction (Nimmo & Houston, 1978) during starvation, and

Fig. 2. Effects of corticotropin injection on the activities of the soluble PAP (a) and on lipoprotein lipase (b) in rat adipose tissue

Male rats were fed on the starch (▲) and corn-oil (○) diets for 3 weeks (Lawson *et al.*, 1981). They were then injected with 1 unit of corticotropin/kg body wt. and killed at the time shown (Lawson *et al.*, 1981). Results are means $\pm$ s.d. for eight rats at time = 0, or for four rats at other time points. Similar changes in these two activities were found in the other dietary groups described in the present paper (see the text).

Table 2. *The effects of starvation on the activities of various enzymes in rat adipose tissue*

Rats were starved for the times shown. Enzyme activities were determined as in Table 1 and are expressed as means $\pm$ S.D. for the numbers of rats shown in parentheses. The significance of the difference between groups is indicated as described in the legend to Table 1. The microsomal GPAT activity was assumed to be the difference between total and *N*-ethylmaleimide-insensitive GPAT activities.

Enzyme activity	Product formed (nmol/min per mg of protein)		
	Control (I)	24 h-starved (II)	48 h-starved (III)
Acyl-CoA synthetase	41.2 $\pm$ 7.4 (12) I versus II†	30.8 $\pm$ 6.6 (12)	34.9 $\pm$ 6.6 (4)
Mitochondrial GPAT	1.05 $\pm$ 0.21 (11)	1.05 $\pm$ 0.54 (12) II versus III†	0.521 $\pm$ 0.175 (12) I versus III††
Microsomal GPAT	4.12 $\pm$ 1.36 (11)	4.75 $\pm$ 1.22 (12)	4.70 $\pm$ 0.97 (12)
<i>N</i> -Ethylmaleimide-insensitive DHAPAT	0.076 $\pm$ 0.037 (8)	0.047 $\pm$ 0.0019 (7)	0.032 $\pm$ 0.0089 (7) I versus III***
Soluble PAP	33.9 $\pm$ 5.8 (4)	38.3 $\pm$ 10.2 (4)	32.5 $\pm$ 6.5 (4)
Diacylglycerol acyltransferase	1.01 $\pm$ 0.16 (4)	0.96 $\pm$ 0.13 (4)	1.19 $\pm$ 0.44 (4)
Ethanol acyltransferase	0.27 $\pm$ 0.040 (4)	0.28 $\pm$ 0.067 (4)	0.31 $\pm$ 0.14 (4)
	Fatty acid formed (μ mol/min per g of tissue)		
Lipoprotein lipase	2.33 $\pm$ 1.22 (4)	0.89 $\pm$ 0.34 (4) I versus II††	0.65 $\pm$ 0.11 (4) I versus III††

that post-mortem alterations in the extent of this phosphorylation might obscure some decreases in activity. EDTA (5mM) and NaF (20mM) were therefore added to the medium used for homogenizing one fat-pad, whereas these were omitted from that used for the other fat-pad. The activities of acyl-CoA synthetase, total GPAT, DGAT and ethanol acyltransferase in starved rats relative to those in the fed controls were not significantly altered by EDTA and NaF. Similarly, 5mM-EDTA alone in the medium did not change the relative activities of PAP. NaF was not included in this instance, since it inhibits PAP.

Discussion

No deacylation of phosphatidate was detected in either microsomal or soluble fractions of adipose tissue under conditions where this could be readily demonstrated in liver fractions. It is therefore unlikely that the deacylation-reacylation cycle that has been proposed as a possible mechanism in controlling triacylglycerol synthesis in liver could operate in adipose tissue. PAP activity in adipose tissue did not increase 6h after the ingestion of fructose, sorbitol, glycerol or ethanol, whereas increases were seen in hepatic PAP activity in the same rats (Sturton *et al.*, 1978). The latter increase was partly mediated by the increased concentrations of serum corticosterone that occurred in the absence of an insulin response (Brindley *et al.*, 1979b). The injection of corticotropin stimulated corticosterone release, but over a 6h period it also did not alter insulin concentrations (Lawson *et al.*,

1981). This treatment was accompanied by a decrease after 15 min in the activities of LPL and soluble PAP (Fig. 2), whereas there were no significant short-term changes in the soluble PAP activity in the livers of the same animals at this early period (Lawson *et al.*, 1981). The PAP activity subsequently increased at 6h by 3.3–4.3-fold in the liver (Lawson *et al.*, 1981), whereas that in adipose tissue returned to this original value at this time (Fig. 2). The activity of LPL remained lower 6h after corticotropin injection (Fig. 2), but this time should have coincided with the minimum activity point of the circadian rhythm (de Gasquet *et al.*, 1977). The short-term decreases in the activities of LPL and PAP were reproducibly seen during November–December, but the differences were not statistically significant when the experiments were performed from March to August. This discrepancy can probably be related to the observation that the lipolytic effects of corticotropin were much higher in rats from October to early February, whereas little effect was seen from March to August (Boright *et al.*, 1962).

Corticotropin itself may have been responsible for the decrease in PAP and LPL activities (Fig. 2), and this hormone has decreased LPL activity in adipose tissue *in vitro* (Wing *et al.*, 1966; Nestel & Austin, 1969). Alternatively other hormones associated with stress could have been involved. For example, catecholamines produce rapid decreases in the activity of LPL (Ashby *et al.*, 1978, 1979) and PAP (Saggerson *et al.*, 1979) in adipose tissue, or adipocytes *in vitro*.

Starvation should also have stimulated lipolysis relative to fatty acid uptake and esterification by

adipose tissue. Of the enzymes assayed, LPL showed the most marked decreases in activity especially at 24 h of starvation (Table 2). The only significant decreases in the activities of enzymes involved in fatty acid esterification were those of acyl-CoA synthetase after 24 h, and of the mitochondrial GPAT and the *N*-ethylmaleimide-insensitive DHAPAT after 48 h. It is not clear whether the lower activities of the mitochondrial GPAT and the *N*-ethylmaleimide-insensitive DHAPAT are related to a decreased ability to synthesize triacylglycerols. At present the consensus of opinion is that the most important route of phosphatidate synthesis for this pathway is via the GPAT in the endoplasmic reticulum. It is possible that the mitochondrial GPAT may be more involved in regulating the oxidation of fatty acids by competing for acyl-CoA esters. The physiological function of the ethanol acyltransferase is also unclear and it was essentially assayed as a by-product of the diacylglycerol acyltransferase assay.

LPL also showed the greatest changes in activity of the enzymes investigated in rats fed on the different diets (Table 1). This activity was much higher in the adipose tissue of rats fed on the high-carbohydrate rather than on the high-fat diets. The reverse was true in heart (Table 1). These results agree with those of Weisenburg-Delorme & Harris (1975) and Childs *et al.* (1981). The former authors also demonstrated that LPL activity in skeletal muscle changed in a similar way to that in heart. LPL activity was also lower in the adipose tissue of rats fed on the sucrose rather than on the starch diet (Table 2). These decreases in the potential of adipose tissue to take up fatty acids from very-low-density lipoproteins could contribute to the increase in circulating triacylglycerols that were seen in the rats fed on the sucrose, corn-oil or beef-tallow diets (Lawson *et al.*, 1981).

Rats fed on high-fat diets can exhibit insulin-insensitivity in adipose tissue (for discussion see Brindley *et al.*, 1981) and to a lesser extent high-sucrose diets may have this effect (Hallfrisch *et al.*, 1978). The activity of LPL in adipose tissue is largely controlled by insulin, and glucocorticoids enhance insulin action (Ashby *et al.*, 1979). An insensitivity to insulin could, therefore, explain the lower LPL activities in adipose tissue of rats fed on the sucrose, corn-oil and beef-tallow diets (Table 2). By contrast, the LPL activity in heart is mainly controlled by glucocorticoid availability (de Gasquet *et al.*, 1975; Friedman *et al.*, 1978). Rats fed on high-fat diets can show exaggerated glucocorticoid responses (Brindley *et al.*, 1981) and feeding triacylglycerol to rats has been reported to increase the concentrations of circulating glucocorticoids (Lenz *et al.*, 1970). The basal concentrations of circulating insulin and corticosterone were not

significantly different (Lawson *et al.*, 1981) at the time when LPL activity was determined (Table 2). However, this was at the beginning of the light period, which is not the period of maximum feeding. It is possible that changes in hormonal balance and sensitivity could be responsible for the relative changes in heart and adipose-tissue LPL activities that are shown in Table 1.

Differences in hormonal status could also account for why feeding a diet rich in rape-seed oil increased LPL activity in adipose tissue (Hülsmann *et al.*, 1979). However, the rats used in the latter experiments were starved for 1 day, and then fed for 4 days on a diet in which erucic acid comprised 43% of the fatty acids. Feeding with a diet rich in corn oil, rather than beef tallow, has also been reported to increase LPL activity in adipose tissue (Cryer *et al.*, 1978). In these experiments guinea pigs were used and they were weaned on to the experimental diets.

Differences have been observed concerning the effects of dietary modification on the activity of GPAT. Dodds *et al.* (1976) reported that feeding on a diet rich in starch rather than sucrose, beef tallow or corn oil increased the activity of GPAT in adipose tissue. By contrast, Table 1 shows that this activity was higher in rats fed on beef tallow than in those fed on the starch diet. Part of this discrepancy could have arisen because Dodds *et al.* (1976) did not maintain a constant-energy density relative to protein in their dietary modifications.

The present work emphasizes that the control of the activity of enzymes involved in triacylglycerol synthesis in adipose tissue differs from that in liver. There is some evidence that these activities in adipose tissue are subject to long-term control (Tables 1 and 2). However, the largest changes in this respect were those of LPL, which controls the uptake of fatty acids from very-low-density lipoproteins and chylomicrons. This enzyme also appears to respond to relatively short-term control in adipose tissue (Fig. 2). In addition, evidence was presented that PAP may also respond *in vivo* to this type of regulation.

This work was supported by grants from M.R.C. and Servier Laboratories. The S.R.C. provided a studentship to A. D. P. and CASE awards to R. J. J. and N. L. The skilful technical assistance of Miss S. L. Burditt, Mrs. C. Finnerty and Mrs. I. S. McKechnie is gratefully acknowledged.

References

- Ashby, P., Bennett, D. P., Spencer, I. M. & Robinson, D. S. (1978) *Biochem. J.* **176**, 865–872.
- Ashby, P., Parkin, S., Walker, K., Bennett, D. P. & Robinson, D. S. (1979) In *Obesity: Cellular and Molecular Aspects* (Ailhaud, G., ed.), vol. 87, pp. 149–158, INSERM, Paris.

- Bakken, A. M., Farstad, M. & Berge, R. (1979) *FEBS Lett.* **99**, 47–50
- Bjerve, K. S., Daae, L. N. W. & Bremer, J. (1974) *Anal. Biochem.* **58**, 238–245
- Boright, H. A., Engel, F. L., Lebovitz, H. E., Kostyo, J. L. & White, J. E. (1962) *Biochem. J.* **83**, 95–101
- Bowley, M., Cooling, J., Burditt, S. L. & Brindley, D. N. (1977) *Biochem. J.* **165**, 447–454
- Bradford, M. M. (1976) *Anal. Biochem.* **72**, 248–254
- Brindley, D. N. (1981) *Clin. Sci.* **61**, 129–133
- Brindley, D. N., Cooling, J. & Burditt, S. L. (1979a) in *Obesity: Cellular and Molecular Aspects* (Ailhaud, G., ed.), vol. 87, pp. 251–262, INSERM, Paris
- Brindley, D. N., Cooling, J., Burditt, S. L., Pritchard, P. H., Pawson, S. & Sturton, R. G. (1979b) *Biochem. J.* **180**, 195–199
- Brindley, D. N., Cooling, J., Glenny, H. P., Burditt, S. L. & McKechnie, I. S. (1981) *Biochem. J.* **200**, 275–283
- Childs, M. T., Tellefson, J., Knopp, R. H. & Bowden, D. A. (1981) *Metab. Clin. Exp.* **30**, 27–35
- Coleman, R. A. & Bell, R. H. (1976) *J. Biol. Chem.* **251**, 4537–4543
- Cryer, A., Kirtland, J., Jones, H. M. & Gurr, M. I. (1978) *Biochem. J.* **170**, 169–172
- Daniel, A. M. & Rubinstein, D. (1968) *Can. J. Biochem.* **46**, 1039–1045
- de Gasquet, P., Pequignot-Planche, E., Tonnu, N. T. & Diaby, F. A. (1975) *Horm. Metab. Res.* **7**, 152–157
- de Gasquet, P., Griglio, S., Pequignot-Planche, E. & Malewiak, M. I. (1977) *J. Nutr.* **107**, 199–212
- Dodds, P. F., Brindley, D. N. & Gurr, M. I. (1976) *Biochem. J.* **160**, 701–706
- Friedman, G., Stein, O. & Stein, Y. (1978) *Biochim. Biophys. Acta* **531**, 222–232
- Hallfrisch, J., Lazar, F. L. & Reiser, S. (1978) *Nutr. Rep. Int.* **18**, 359–367
- Hosaka, K., Yamashita, S. & Numa, S. (1975) *J. Biochem. (Tokyo)* **77**, 501–509
- Hülsmann, W. C., Geelhoed-Mieras, M. M., Jansen, H. & Houtsmuller, U. M. T. (1979) *Biochim. Biophys. Acta* **572**, 183–187
- Jamdar, S. C. & Fallon, H. J. (1973) *J. Lipid Res.* **14**, 517–524
- Jason, C. J., Polokoff, M. A. & Bell, R. M. (1976) *J. Biol. Chem.* **251**, 1488–1492
- Lawson, N., Jennings, R., Pollard, A. D., Sturton, R. G., Ralph, S. J., Marsden, C. A., Fears, R. & Brindley, D. N. (1981) *Biochem. J.* **200**, 265–273
- Lehtonen, M. A., Savolainen, M. J. & Hassinen, I. E. (1979) *FEBS Lett.* **99**, 162–165
- Lenz, P. H., Wien, G. H. & Fleischman, A. I. (1970) *Lipids* **5**, 524–530
- Lloyd-Davies, K. A. & Brindley, D. N. (1975) *Biochem. J.* **152**, 39–49
- Manning, R. & Brindley, D. N. (1972) *Biochem. J.* **130**, 1003–1012
- McKeel, D. W. & Jarrett, L. (1970) *J. Cell Biol.* **44**, 417–432
- Moller, F., Green, P. & Harkness, E. J. (1977) *Biochim. Biophys. Acta* **486**, 359–368
- Nestel, P. J. & Austin, W. (1969) *Life Sci.* **8**, 157–164
- Nilsson-Ehle, P. & Schotz, M. C. (1976) *J. Lipid Res.* **17**, 536–541
- Nimmo, H. G. & Houston, B. (1978) *Biochem. J.* **176**, 607–610
- Robinson, D. S. (1970) *Comp. Biochem.* **18**, 51–116
- Roncari, D. A. K. & Hollenberg, C. H. (1967) *Biochim. Biophys. Acta* **137**, 446–463
- Saggerson, E. D., Sooranna, S. R. & Cheng, C. H. K. (1979) in *Obesity: Cellular and Molecular Aspects* (Ailhaud, G., ed.), vol. 87, pp. 223–237, INSERM, Paris
- Saggerson, E. D., Carpenter, C. A., Cheng, C. H. K. & Sooranna, S. R. (1980) *Biochem. J.* **190**, 183–189
- Salaman, M. R. & Robinson, D. S. (1966) *Biochem. J.* **99**, 640–647
- Schlossman, D. M. & Bell, R. M. (1976) *J. Biol. Chem.* **251**, 5738–5744
- Shapiro, B. & Tzur, R. (1968) *Ann. N.Y. Acad. Sci.* **149**, 784–790
- Sooranna, S. R. & Saggerson, E. D. (1979) *FEBS Lett.* **99**, 67–69
- Sturton, R. G. & Brindley, D. N. (1978) *Biochem. J.* **171**, 263–266
- Sturton, R. G. & Brindley, D. N. (1980) *Biochim. Biophys. Acta* **619**, 494–505
- Sturton, R. G., Pritchard, P. H., Han, L.-Y. & Brindley, D. N. (1978) *Biochem. J.* **174**, 667–670
- Tornqvist, H. & Belfrage, P. (1976) *J. Lipid Res.* **17**, 542–545
- Weisenburg-Delorme, C. L. W. & Harris, K. L. (1975) *J. Nutr.* **105**, 447–451
- Wing, D. R., Salaman, M. R. & Robinson, D. S. (1966) *Biochem. J.* **99**, 648–656