Effects of chronic modification of dietary fat and carbohydrate in rats

The activities of some enzymes of hepatic glycerolipid synthesis and the effects of corticotropin injection

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1. Rats were fed on diets enriched with starch, sucrose, corn oil or beef tallow for 3 weeks and the activities of various enzymes in the liver were measured. 2. The mitochondrial glycerol phosphate acyltransferase activity was lower in rats fed on the starch diet than on the two high-fat diets. 3. The non-microsomal (presumably peroxisomal) dihydroxyacetone phosphate acyltransferase activity was higher in rats fed on the starch diet and corn-oil diets than in those fed on the sucrose and beef-tallow diets. Urate oxidase activity was higher in rats fed on the starch diet than in the three other groups. There were no significant differences in the activity of acyl-CoA oxidase among the groups. 4. The activity of soluble phosphatidate phosphohydrolase was not significantly different among the dietary groups. There were increases of 3.3–4.3-fold in this activity in the dietary groups 6 h after injection of corticotropin. The equivalent increases for the mitochondrial glycerol phosphate acyltransferase were 1.4–1.6 fold. 5. The corticosterone responses to the corticotropin injection were not significantly different between dietary groups. However, the corticosterone response of the rats fed on the two high-fat diets was prolonged when the rats were given an acute load of fructose [Brindley, Cooling, Glenny, Burditt & McKechnie (1981) *Biochem. J.* **200**, 275–283]. 6. Rats fed on the high-fat diets had higher concentrations of circulating cholesterol than those fed on the starch and sucrose diets. Serum triacylglycerol concentrations were lower in the rats fed on the starch diet than in the three other groups. 7. The results are discussed in terms of the relationship between diet, hormonal balance and hepatic glycerolipid metabolism.

The work in this paper was designed to investigate the interrelation between dietary composition and the control of hepatic triacylglycerol synthesis, particularly by glucocorticoids. It is known that these hormones stimulate the synthesis and secretion of triacylglycerols by the liver (Klausner & Heimberg, 1967; Reaven *et al.*, 1974; Glenny & Brindley, 1978). Glucocorticoids also increase the activity of PAP, which is considered to be a regulatory enzyme in this process (Glenny & Brindley, 1978; Brindley *et al.*, 1979a,b; Lehtonen *et al.*, 1979; Brindley, 1981). It has been reported

that feeding high-fat diets to rats increased their stress reactions to cold (Carroll & Noble, 1952) and their production of corticosterone in response to nembutal narcosis and to corticotropin injection (Hülsmann, 1978). This indicated that high-fat diets can increase the sensitivity or capacity of the adrenal gland to secrete corticosterone. Adrenal hypertrophy has been reported after this type of dietary modification (Carroll & Noble, 1952; Blázquez *et al.*, 1971).

In the present experiments rats were fed on diets enriched with starch, sucrose, corn oil or beef tallow, since there is considerable interest in the cardiovascular effects of different types of carbohydrate and fat. The effects of this chronic dietary modification on body composition, the concentration of some hormones and metabolites in blood and the

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

activity of enzymes involved in glycerolipid synthesis in the liver were measured. This was done to see whether any changes in these parameters could be related to dietary-induced alterations in glucocorticoid metabolism.

Materials and methods

Materials

The source of most of these has already been described (Sánchez *et al.*, 1973; Sturton & Brindley, 1977). Fructose 1,6-bisphosphate, aldolase (EC 4.1.2.13), triose phosphate isomerase (EC 5.3.1.1), 4-methylpyrazole, semicarbazide hydrochloride and acetylacetone were purchased from the Sigma (London) Chemical Co., Poole, Dorset, U.K. All radiochemicals were obtained from The Radiochemical Centre, Amersham, Bucks., U.K., and all assay kits were purchased from Boehringer Corp. (London), Lewes, East Sussex, U.K. Sterile solutions of β -corticotropin-(1-24)-peptide (Synacthen; 0.25 mg, 25 units/ml) were obtained from Ciba Laboratories, Horsham, West Sussex, U.K.

Animals

Male Wistar rats from the University of Nottingham Joint Animal Breeding Unit were accommodated at 22°C in a room that was lit from 08:00 h to 20:00 h. They were initially maintained on a 41B diet obtained from Wherry & Son, Bourne, Lincolnshire, U.K. This diet consisted of about 14% (by weight) of digestible protein, 45% of digestible carbohydrate and 3% of fat. The rats were housed, four to a cage, and they were transferred to grid-bottomed cages 5 days before the dietary modification. Each experiment involved four groups of rats that were then fed *ad libitum* on one of the experimental diets described in Table 1. Weight gains and average food intakes for groups of rats were determined on every other day. After 3 weeks some groups of rats were killed with minimum stress between 09:30 h and 10:30 h by decapitation with a guillotine. Blood samples were collected from the necks and tissues were taken for analysis (Brindley *et al.*, 1979b). Serum was prepared immediately from the blood and then frozen and stored at -20°C until required. Alternatively, some of the rats were

Table 1. *Composition of diets*

The four experimental diets were specially prepared in pelleted form by B.P. Nutrition (U.K.), Stepfield, Withern, Essex CM8 3AB, U.K. The vitamin and mineral premixes provided the requirements for rats as described by Clarke *et al.* (1977). Metabolizable energy and the relative energy contributions of fat, protein and carbohydrate (excluding fibre) were estimated by using the values of 37, 17 and 16 kJ/g respectively as recommended by McCance & Widdowson (1978).

	Starch diet (I)	Sucrose diet (II)	Corn-oil diet (III)	Tallow diet (IV)
(a) Formulae (g·kg ⁻¹)				
Vitamin premix	10	10	10	10
Mineral premix	10	10	10	10
U.D. Yeast	10	10	10	10
Skimmed-milk powder	30	30	40	40
Fish meal	185	185	200	200
Oat feed	145	145	160	160
Wheat bran	100	100	125	125
Wheat	100	100	160	150
Sucrose	—	400	—	—
Cornstarch	400	—	85	65
Beef tallow	—	—	—	220
Corn oil	10	10	200	10
(b) Calculated composition (g·kg ⁻¹)				
Fat	30	30	223	230
Protein	165	165	190	188
Carbohydrate	580	580	350	345
Fibre	55	55	67	67
Ash	70	70	70	70
(c) Estimated metabolizable energy (MJ·kg ⁻¹)	13.20	13.20	17.08	17.23
(d) Relative contribution to metabolizable energy (%)				
Fat	8	8	48	49
Protein	21	21	19	19
Carbohydrate	70	70	33	32

injected intramuscularly into the hind leg with 1 unit of corticotropin/kg body wt. The injection material was prepared by diluting Synacthen (0.25 mg, equivalent to 25 units/ml) to a concentration of 1 unit/ml with sterile 0.15 M-NaCl. The rats were then killed as before at an appropriate time after this injection. Preliminary experiments with rats maintained on the 41B diet showed that this dose of corticotropin produced the maximum corticosterone response after 30 min. At this time the prior injection of 0.15 M-NaCl alone increased the corticosterone concentration to 0.60 ± 0.07 nM (mean $\pm$ S.E.M. from four rats). The use of the saturating dose of corticotropin ensures that the results are not complicated by differences in the stress reaction to the injection itself that may occur between dietary groups.

Preparation of homogenates and soluble fractions of rat liver

Rat livers were homogenized with a Teflon/glass homogenizer in 30 ml of 0.3 M-sucrose containing 0.5 mM-dithiothreitol and adjusted to pH 7.4 with KHCO_3 . The homogenate was filtered through a coarse nylon mesh. The soluble fraction was prepared by centrifuging the homogenate for 90 min at 4°C and 76 000 g ($r_{av.} = 7.62$ cm), and samples of the supernatant were dialysed for about 16 h at 4°C against 100 vol. of 0.3 M-sucrose containing 0.5 mM-dithiothreitol.

Determination of the serum concentrations of insulin and corticosterone

These were performed as described by Brindley *et al.* (1979b) with the exception that corticosterone was only assayed by the displacement of [^3H]-corticosterone from the binding globulin.

Determination of tryptophan concentrations

Total and free tryptophan concentrations were determined in serum as described by Bloxham & Warren (1974). Albumin-bound tryptophan was separated from free tryptophan by ultrafiltration, without pH control, by using CF25 Amicon Diaflo membrane cones (Curzon & Knott, 1975).

Determination of fatty acid concentrations

These were determined by an automated method (Elphick, 1975).

Determination of DNA and protein concentrations

These are determined as described by Hübscher *et al.* (1965).

Determination of the concentrations of glycerol and triacylglycerol

Glycerol was determined by a linked enzyme reaction based on a Test Combination Kit that was

purchased from The Boehringer Corp. (London). The method was modified by Dr. M. Elphick and the sensitivity thereby increased by employing a spectrofluorimeter to determine NADH disappearance. Serum samples (20 μl) were treated with 700 μl of 0.28 M- HClO_4 and the precipitate was removed by centrifugation. Part of the supernatant (500 μl) was added to 200 μl of 0.81 M-KOH and after cooling for 10 min on ice the precipitate was removed by centrifugation. Samples (500 μl) of the clear extract were added to 1 ml of 0.25 M-triethanolamine buffer, adjusted to pH 7.6 with HCl and containing 6 mM- MgSO_4 . To this solution was added 5 μl of 0.5 M-triethanolamine, adjusted to pH 9.4 with HCl and containing 6 mM- MgSO_4 , 6.7 mM-NADH, 33 mM-ATP and 11 mM-phosphoenolpyruvate. Lactate dehydrogenase (4 units) and pyruvate kinase (0.65 unit) were then added in a mixed solution of 5 μl . The fluorescence of the final assay medium was monitored continuously at 457 nm by using a Vitatron fluorimeter with an excitation wavelength of 366 nm. After 10 min the fluorescence stabilized and 5 μl of a solution of glycerokinase (0.75 unit) was added. The maximum fluorescence change attributed to the addition of glycerol kinase was used as a measurement of glycerol concentration. Standards of glycerol (0.1–0.8 mM) were taken through the same extraction procedure and assayed. The fluorimeter was calibrated for each measurement by adjusting the fluorescence to 80% with a 0.22 $\mu\text{g/ml}$ solution of quinine sulphate in 0.05 M- H_2SO_4 .

For the determination of triacylglycerol 0.1 ml samples were placed in small glass tubes and 0.25 ml of 0.5 M-KOH in 90% (v/v) ethanol was added. The tubes were sealed and heated at 60°C for 2 h. The tubes were cooled on ice and 0.1 ml of 0.15 M- MgSO_4 was added with vigorous shaking. The precipitate was removed by centrifugation and the supernatant was transferred to another tube. Samples of the supernatant (25 μl) were added to 0.475 ml of water and 1 ml of 0.25 M-triethanolamine, adjusted to pH 7.6 with HCl and containing 6 mM- MgSO_4 , was added. The concentration of glycerol was measured as described above and the triacylglycerol concentration was calculated by subtracting the concentration of the free glycerol from that of the total glycerol after hydrolysis.

Determination of glucose and cholesterol concentrations

These were assayed by using commercially available kits. High-density-lipoprotein cholesterol was determined after precipitating other lipoproteins with 92 mM- MnCl_2 and 1.3 mg of heparin/ml as recommended by Warnick & Albers (1978).

Enzyme assays

These were performed under optimum conditions and the mean activity of each sample was calculated from rates obtained at three different protein concentrations.

Determination of microsomal, mitochondrial and total GPAT activities

Each assay contained, in a final volume of 0.25 ml: 250 mM-KCl, 50 mM-Tris buffer, adjusted to pH 7.4 with HCl, 0.7 mM-dithiothreitol, 5 mM-*sn*-[1,3-³H]glycerol 3-phosphate (sp. radioactivity 1 Ci/mol), 136 μ M-palmitoyl-CoA, 6 mg of fatty acid-poor bovine serum albumin/ml and 300–900 μ g of homogenate protein. The reaction was stopped after 3 min at 37°C by adding 1 ml of water-saturated butanol followed by 0.75 ml of butanol-saturated water. The phases were separated by centrifugation and the butanol phase was then washed twice with 1 ml portions of butanol-saturated water. Radioactivity in the bottom phase was determined by liquid-scintillation counting. This gives the total GPAT activity of the homogenate. Portions of the homogenate were also incubated for 10 min at 4°C with 10 mM-*N*-ethylmaleimide. The excess of this compound was then removed by adding 10 mM-dithiothreitol. The use of *N*-ethylmaleimide inhibits the GPAT activity of the endoplasmic reticulum, whereas that in the mitochondria is not affected (Yamada & Okuyama, 1978; Nimmo, 1979; Bates & Saggerson, 1979; Haldar *et al.*, 1979). This latter activity was determined as described above and the microsomal activity was calculated as the difference between this and the total GPAT activity. Reaction conditions were optimized in the absence of *N*-ethylmaleimide, and the rates were proportional to the amount of enzyme added when *N*-ethylmaleimide was present or absent.

Determination of 'peroxisomal' DHAPAT activity

This was assayed by a method based on that of Bates & Saggerson (1979). Each assay contained, in a final volume of 0.25 ml: 120 mM-KCl, 50 mM-Tris, adjusted to pH 7.4 with HCl, 4 mM-MgCl₂, 8 mM-NaF, 136 μ M-palmitoyl-CoA, 0.25 mM-[U-¹⁴C]-fructose 1,6-bisphosphate (sp. radioactivity 0.8 Ci/mol), 12.5 μ g of aldolase (0.11 unit), 0.75 μ g of triose phosphate isomerase (3.75 units) and 4 mg of fatty acid-poor bovine serum albumin/ml. A 180 μ l portion of the reaction mixture was pre-incubated for 16 min at 30°C to generate the dihydroxy-[¹⁴C]acetone phosphate and the reaction was started with 70 μ l of homogenate containing 300–900 μ g of protein. The esterification reaction was then stopped, after incubation for 8 min at 30°C, by adding 1.88 ml of chloroform/methanol (1:2, v/v) and the lipid product was extracted (Hajra *et al.*, 1968). The

bottom phase was washed twice with synthetic top phase and radioactivity was determined, after evaporating the chloroform, by liquid-scintillation counting.

The homogenate used for these determinations was frozen and stored at –20°C before being used and under these conditions DHAPAT activity showed no latency and there was no stimulation by Triton X-100 and sodium deoxycholate. The homogenate was routinely incubated for 5 min at 4°C with 5 mM-*N*-ethylmaleimide and then used to start the esterification reaction. This procedure inhibited the microsomal GPAT, which can also use dihydroxyacetone phosphate as an acyl-acceptor (Schlossman & Bell, 1976, 1977) and it stimulated the DHAPAT activity by about 30%. This type of stimulation has been reported previously (Bates & Saggerson, 1979; Jones & Hajra, 1980). This assay procedure should predominantly measure the activity of the DHAPAT that is located in the peroxisomal fraction (Hajra *et al.*, 1979; Bates & Saggerson, 1979; Jones & Hajra, 1980), although some DHAPAT that is insensitive to *N*-ethylmaleimide could occur in other organelles.

Determination of soluble PAP activity

PAP activity was determined by the method of Brindley *et al.* (1979b) except that the buffer used was 50 mM-Tris adjusted to pH 7.4 with HCl.

Determination of urate oxidase activity (EC 1.7.3.3)

This was measured by the method of Leighton *et al.* (1968).

Determination of acyl-CoA oxidase activity

This enzyme activity was determined by a method based on that of Thomas *et al.* (1980). Each incubation contained, in a volume of 750 μ l: 50 mM-potassium phosphate buffer, pH 7.4, 523 μ M-palmitoyl-CoA, 50 mM-methanol, 0.5 mM-4-methylpyrazole, 2 mM-semicarbazide and up to 300 μ g of homogenate protein. The tubes were incubated for 10 min at 37°C, the reactions stopped with 250 μ l of 30% (w/v) trichloroacetic acid and formaldehyde was determined by the method of Nash (1953). The addition of up to 100 μ M-FAD⁺ and 1 unit of catalase activity did not alter the rate of formaldehyde formation and these were not included in routine enzyme assays.

Results

Effects of dietary modification on the growth and body composition of rats

The rats in the four groups did not have significantly different weights at the beginning of the experiments and they gained weight steadily throughout the period of dietary modification. The

initial gain in weight in the four groups was not significantly different from that which would have been expected had they been maintained on the standard 41B diet. The overall gain in body weight of the rats fed sucrose was lower than that in the starch-fed group, and the final body weight of the sucrose group was 4% and 3% lower than those for the groups fed on starch and tallow diets respectively (Table 2). This difference was reflected in the lower energy intake estimated for the rats fed on sucrose compared with the three other groups. The rats given the high-fat diets had the highest energy consumption, but this did not result in higher body or fat-pad weights compared with rats fed on the starch diet.

The weights of the livers were lower in the rats fed on the two high-fat diets compared with the high-carbohydrate-diet-fed groups, particularly relative to the rats fed on sucrose. The tallow-diet-fed group had the lowest liver weights when expressed relative to body weight. The protein content of the liver of rats fed on sucrose, and the DNA content of those given the starch diet were higher than for the rats given the high-fat diets. Heart weights were not

significantly different, but the starch-fed group seemed to be lower than the corn-oil-fed group when results were expressed relative to body weight.

Effects of diet on the serum concentrations of insulin, corticosterone and some metabolites

The concentrations of circulating glucose and glycerol were lower in rats fed on sucrose rather than on the corn-oil diet (Table 3). Concentrations of unesterified fatty acids and total cholesterol were higher in rats fed on high-fat rather than high-carbohydrate diets. The concentrations of high-density-lipoprotein cholesterol were higher in rats fed on corn oil than in those given the starch and sucrose diets. However, the relative concentration of total to high-density-lipoprotein cholesterol were not significantly different among the groups. There were also no significant differences between the concentrations of total tryptophan, free tryptophan, insulin and corticosterone. The duration and magnitude of the corticosterone responses to corticotropin injection were not significantly different in the four dietary groups, or in rats given the standard 41B diet (Fig. 1). There were no significant changes

Table 2. *Effects of diet on the food consumption, growth and tissue of rats*

Male rats were fed for 3 weeks on the diets shown in Table 1. Results are means $\pm$ S.D. for the numbers of rats shown in parentheses. Significant differences between groups were calculated by using an unpaired *t* test and they are shown by: *, $P < 0.05$; **, $P < 0.02$; ***, $P < 0.01$; †, $P < 0.005$; ††, $P < 0.001$.

	Starch diet (I)	Sucrose diet (II)	Corn-oil diet (III)	Tallow diet (IV)
Final wt. of rats (g)	265 $\pm$ 22 (56) I versus II*	254 $\pm$ 28 (56)	257 $\pm$ 21 (56)	263 $\pm$ 19 (56) IV versus II*
Wt. gain of rats over previous 21 days (g $\cdot$ 100 g ⁻¹)	35.2 $\pm$ 7.7 (56) I versus II*	31.6 $\pm$ 9.4 (56)	32.4 $\pm$ 7.5 (56)	34.0 $\pm$ 7.7 (56)
Food intake [kJ $\cdot$ (kg body wt.) ⁻¹ $\cdot$ day ⁻¹]	1280 $\pm$ 190 (37) I versus II*	1170 $\pm$ 130 (37) II versus III††† II versus IV†††	1480 $\pm$ 250 (35) I versus III†††	1523 $\pm$ 260 (37) I versus IV†††
Liver wt. (g)	11.1 $\pm$ 0.9 (36) I versus II*	11.7 $\pm$ 1.3 (36) II versus III††† II versus IV†††	10.6 $\pm$ 1.1 (36) I versus III*	10.5 $\pm$ 1.0 (36) I versus IV***
(Liver wt./body wt.) $\times$ 100	4.08 $\pm$ 0.26 (36) I versus II†††	4.37 $\pm$ 0.31 (36) II versus III††† II versus IV†††	4.06 $\pm$ 0.19 (36) III versus IV**	3.91 $\pm$ 0.29 (36) I versus IV**
Liver protein (mg $\cdot$ liver ⁻¹)	1656 $\pm$ 155 (36)	1706 $\pm$ 183 (35)	1600 $\pm$ 168 (36) II versus III**	1603 $\pm$ 164 (36) II versus IV**
Liver DNA (mg $\cdot$ liver ⁻¹)	21.2 $\pm$ 2.45 (35)	20.8 $\pm$ 3.72 (35)	19.5 $\pm$ 2.84 (35) I versus III**	19.4 $\pm$ 2.88 (34) I versus IV**
Heart wt. (g)	0.869 $\pm$ 0.077 (28)	0.869 $\pm$ 0.092 (28)	0.848 $\pm$ 0.065 (28)	0.862 $\pm$ 0.063 (28)
(Heart wt./body wt.) $\times$ 100	0.31 $\pm$ 0.02 (28) I versus III†††	0.32 $\pm$ 0.02 (28)	0.33 $\pm$ 0.02 (28)	0.32 $\pm$ 0.02 (28)
Wt. of epididymal fat-pads (g)	3.60 $\pm$ 0.54 (36) I versus II†	3.22 $\pm$ 0.54 (36)	3.67 $\pm$ 0.85 (36) II versus III***	3.65 $\pm$ 0.71 (36) II versus IV***
(Wt. of fat pads/body wt.) $\times$ 100	1.31 $\pm$ 0.16 (36)	1.26 $\pm$ 0.24 (36) II versus III*	1.39 $\pm$ 0.28 (36)	1.35 $\pm$ 0.25 (36)
Fat-pad protein (mg)	11.8 $\pm$ 1.7 (34) I versus IV†	11.1 $\pm$ 2.0 (35)	11.8 $\pm$ 2.1 (36) III versus IV***	10.5 $\pm$ 1.7 (35)

Table 3. *Effect of diet on the concentration of insulin, corticosterone and metabolites in the blood serum*

Male rats were fed for 3 weeks on the diets shown in Table 1 and the concentrations of hormones and metabolites in the serum are given as means $\pm$ S.D. The number of rats are shown in parentheses and the significance of the differences between groups is indicated as described in the legend to Table 2.

	Starch diet (I)	Sucrose diet (II)	Corn-oil diet (III)	Tallow diet (IV)
[Glucose] (mM)	7.01 $\pm$ 0.77 (8)	6.38 $\pm$ 0.57 (8) II versus III**	7.07 $\pm$ 0.37 (8)	6.89 $\pm$ 1.13 (8)
[Glycerol] (mM)	0.30 $\pm$ 0.10 (8)	0.23 $\pm$ 0.06 (8)	0.30 $\pm$ 0.07 (8) II versus III*	0.34 $\pm$ 0.12 (7) II versus IV*
[Unesterified fatty acid] (mM)	0.25 $\pm$ 0.03 (4) I versus III**	0.28 $\pm$ 0.07 (4) II versus III*	0.44 $\pm$ 0.11 (4)	0.33 $\pm$ 0.01 (4) I versus IV†
[Total tryptophan] (μ M)	112 $\pm$ 24 (8)	110 $\pm$ 12 (8)	123 $\pm$ 23 (8)	127 $\pm$ 23 (8)
[Free tryptophan] (μ M)	11.4 $\pm$ 9.6 (8)	8.3 $\pm$ 7.9 (8)	11.6 $\pm$ 7.3 (8)	11.6 $\pm$ 8.7 (8)
[Triacylglycerol] (mM)	0.88 $\pm$ 0.20 (8)	1.43 $\pm$ 0.52 (8) I versus II† I versus III††	1.63 $\pm$ 0.32 (8) I versus III††	1.47 $\pm$ 0.12 (8) I versus IV††
[Total cholesterol] (mM)	2.04 $\pm$ 0.47 (12) I versus III†	2.14 $\pm$ 0.47 (15) II versus IV†	2.80 $\pm$ 0.66 (13) II versus III†	2.78 $\pm$ 0.48 (11) I versus IV†
[High-density-lipoprotein cholesterol] (mM)	0.78 $\pm$ 0.25 (8) I versus III††	0.92 $\pm$ 0.24 (8) II versus III††	1.20 $\pm$ 0.14 (8)	1.04 $\pm$ 0.26 (8)
[Insulin] (m-units/litre)	66 $\pm$ 31 (8)	49 $\pm$ 18 (8)	89 $\pm$ 78 (8)	56 $\pm$ 36 (8)
[Corticosterone] (nM)	90 $\pm$ 50 (8)	110 $\pm$ 70 (8)	140 $\pm$ 70 (8)	150 $\pm$ 80 (8)

in the concentration of circulating insulin during this 6 h period (results not shown).

Effects of diet and corticotropin injection on the activities of some enzymes involved in hepatic glycerolipid synthesis

Enzyme activities in Table 4 have been expressed relative to DNA to compensate for differences in liver weights among rats. Similar conclusions were also obtained when the activities were expressed relative to protein (cf. Table 2). The total and mitochondrial GPAT activities before and 6 h after corticotropin injection appeared to be lower in rats on the starch diet compared with those fed on high-fat diets, although this was only significant with respect to the corn-oil-fed group (Table 4). Microsomal GPAT activity may have been lower for the starch-fed group than for the corn-oil-fed group, but this only reached significance in rats treated with corticotropin.

An alternative route for synthesizing phosphatidate is by the esterification of dihydroxyacetone phosphate. The 'peroxisomal' DHAPAT activity was higher for the rats given the starch diet than for those given the tallow diet, and it was not significantly different from that in the corn-oil-fed group (Table 4). DHAPAT activity in the rats fed on sucrose was lower than in the rats fed on the starch and corn-oil diets. The activity of urate oxidase, a peroxisomal marker, showed a similar distribution profile, and there was a significant correlation between these two activities ($r=0.463$; $P<0.01$). There was also a significant correlation ($r=0.429$; $P<0.05$) between the activities of

Fig. 1. *Effects of corticotropin injection on the concentration of circulating corticosterone*

Rats were fed for 3 weeks on the starch (○), sucrose (△), corn-oil (●) or beef-tallow (▲) diet, or they were maintained on the standard 41B diet (□). They were injected with 1 unit of corticotropin/kg body wt. and were killed at the times indicated. Corticosterone concentrations are means from three to eight rats per group. Error bars are not shown to simplify the graph.

DHAPAT and acyl-CoA oxidase which is required for the oxidation of fatty acids in peroxisomes. The latter activity did not vary significantly in different dietary groups and the correlation coefficients in themselves do not establish the peroxisomal location of the DHAPAT.

Table 4. *Effect of diets and corticotropin injection on the activities of various enzymes in the liver*

Male rats were fed for 3 weeks on the diets in Table 1. The activities of the enzymes were measured in the absence of a further treatment (section a), or 6 h after the injection of 1 unit of corticotropin·(kg body wt.)⁻¹ (section b). Activities are expressed in nmol of substrate converted·(mg of DNA)⁻¹·min⁻¹ and are means ± s.d. The numbers of rats are shown in parentheses and the significance of the differences between groups is indicated as described in the legend to Table 2.

	Enzyme activity	Starch diet (I)	Sucrose diet (II)	Corn-oil diet (III)	Tallow diet (IV)
(a) No corticotropin injected	Total GPAT	129 ± 29 (8)	208 ± 38 (7)	233 ± 34 (8)	228 ± 47 (7)
		I versus III*			
	Mitochondrial GPAT	105 ± 24 (8)	123 ± 19 (7)	136 ± 23 (8)	136 ± 18 (7)
				I versus III**	I versus IV**
	Microsomal GPAT	87 ± 11 (8)	94 ± 21 (6)	96 ± 28 (8)	94 ± 28 (6)
	Peroxisomal DHAPAT	13.4 ± 1.9 (8)	10.7 ± 1.5 (8)	12.5 ± 1.4 (8)	10.5 ± 1.3 (8)
		I versus II***	II versus III*	III versus IV**	I versus IV†
(b) Corticotropin injected 6 h previously	Soluble PAP	46 ± 13 (8)	50 ± 9 (8)	46 ± 6 (8)	46 ± 12 (8)
	Urate oxidase	2760 ± 650 (8)	2074 ± 397 (8)	2175 ± 335 (8)	1959 ± 247 (8)
		I versus II*		I versus III*	I versus IV*
	Acyl-CoA oxidase	357 ± 75 (8)	338 ± 61 (8)	360 ± 71 (8)	354 ± 41 (8)
	Total GPAT	228 ± 21 (3)	307 ± 71 (4)	310 ± 30 (4)	334 ± 104 (4)
		I versus III**			
	Mitochondrial GPAT	145 ± 16 (3)	199 ± 56 (4)	193 ± 28 (4)	204 ± 56 (3)
		I versus III*			
	Microsomal GPAT	83 ± 6 (3)	108 ± 23 (4)	118 ± 13 (4)	130 ± 49 (3)
		I versus III***			
	Soluble PAP	173 ± 90 (4)	214 ± 66 (4)	153 ± 36 (4)	188 ± 70 (4)

The subsequent incorporation of phosphatidate into phosphatidylcholine, phosphatidylethanolamine and triacylglycerol requires its conversion into diacylglycerol. The activity of the soluble PAP was not significantly different between dietary groups either before, or 6 h after, the injection of corticotropin. This treatment, however, gave an increase in PAP activity of 3.3–4.3-fold ($P < 0.005$), as expected from previous work (Glenny & Brindley, 1978; Lehtonen *et al.*, 1979). The total GPAT was also higher ($P < 0.05$ – 0.005) 6 h after corticotropin injection in the sucrose-, corn-oil- and tallow-fed groups. The mitochondrial GPAT activity was increased ($P < 0.05$ – 0.005) in all four dietary groups. There were no statistically significant increases in the microsomal GPAT activities at 6 h after corticotropin injection (Table 4). None of the GPAT and PAP activities was significantly altered by corticotropin at 15, 30, 100 and 200 min after its injection (results not shown).

The long-term effects of injecting corticotropin were also investigated by using rats maintained on the standard 41B diet. At 6 h after injection the total mitochondrial and microsomal GPAT activities and the DHAPAT activity appeared to be increased by 1.5-, 1.3-, 1.6- and 1.3-fold respectively, compared with untreated rats killed at the same time. However, with four rats in each group these results were not statistically significant. At 9 h after injection the

microsomal GPAT activity was 1.3-fold higher ($P < 0.05$) than in untreated rats, but there was again no statistically significant change in the total and mitochondrial GPAT activities and the DHAPAT activity. It is concluded that some long-term increases in GPAT activity may result from corticotropin injection. However, these increases are very small by comparison with those obtained for PAP.

Discussion

Particular care was taken in the present experiments to ensure that the act of modifying the diet rather than its composition did not produce undue stress to the rats. The diets were based on the formulation of the standard 41B, they were pelleted and 19–21% of the energy was obtained from protein in each case. The weight gains of the rats fed on the starch, corn-oil and beef-tallow diets were not significantly different and the rats fed on sucrose gained only slightly less weight than did those fed on the starch diet. The rats were extensively characterized with respect to their body compositions and to the concentration of metabolites, insulin and corticosterone in their blood sera. This was because the type of dietary modification can profoundly affect glucocorticoid status and metabolic responses (Brindley *et al.*, 1981). Such differences may explain why there are so many apparently contradictory

reports concerning the effects of dietary modification.

Hülsmann (1978) reported that rats given high-fat diets had an increased adrenal capacity to secrete corticosterone when injected with corticotropin. We used the same dose of corticotropin, but failed to demonstrate this difference (Fig. 1). However, the present rats fed the corn-oil and beef-tallow diets did show an increased corticosterone response when they were given a standard load of fructose (Brindley *et al.*, 1981). We do not yet know the mechanism by which fructose produces this response, but it seemed likely that it could be mediated at the level of the hypothalamus and the production of corticoliberin. This release is thought to mediate largely through the action of 5-hydroxytryptamine (Naumenko, 1968; Popova *et al.*, 1972; Fuller *et al.*, 1976; Jones *et al.*, 1976) and the turnover of the latter transmitter in the hypothalamus can increase in rats fed acutely with high-fat diets. This may result from the displacement of tryptophan from albumin by high concentrations of unesterified fatty acids (Curzon & Knott, 1975; Hutson *et al.*, 1976). Although the concentrations of unesterified fatty acids in the blood were higher in the rats fed on the high-fat diets, no significant differences were detected in the serum concentrations of free or total tryptophan (Table 3).

The increased concentrations of circulating triacylglycerol in rats fed on the high-fat diets (Table 3) were accompanied by increases in the activity of the mitochondrial GPAT in liver (Table 4), and decreases in lipoprotein lipase activity in adipose tissue (Lawson *et al.*, 1981). However, at present it is not known whether the changes in GPAT activity are related to the hypertriglyceridaemia, or even whether the phosphatidate produced in mitochondria can be converted into triacylglycerol. The basal microsomal GPAT activities were not significantly different among dietary groups, although the trends were similar to those of mitochondrial GPAT (Table 4). Other reports demonstrate that high-fat diets increase (Fallon & Kemp, 1968; Joly *et al.*, 1973), decrease (Aas & Daae, 1971; Iritani & Fukuda, 1980) or do not alter the microsomal GPAT activity (Wiegand *et al.*, 1973; Glennly *et al.*, 1978; Kako & Peckett, 1981). The microsomal DHAPAT activity is probably identical with the GPAT activity (Schlossmann & Bell, 1976, 1977). This DHAPAT activity has been shown to increase after feeding rats on diets rich in glucose or fructose (Lamb & Fallon, 1976), to decrease in those given unsaturated fat (Rao *et al.*, 1971) or not to change significantly after giving saturated or polyunsaturated fat (Glennly *et al.*, 1978).

The 'peroxisomal' DHAPAT activity was higher in rats given the starch and corn-oil diets than in those fed on the sucrose or beef-tallow diets. We are not aware of other reports on the effects of dietary

modification on this activity. We had thought that giving high-fat diets might have increased this DHAPAT activity, since they can produce peroxisomal proliferation. The latter is associated with an increased acyl-CoA oxidase activity (Osmundsen *et al.*, 1979; Ishii *et al.*, 1980; Neat *et al.*, 1980). However, such proliferation does not appear to be an obligatory consequence of feeding on high-fat diets, since it was not observed in the present experiments.

Feeding rats with diets enriched with glucose, fructose (Lamb & Fallon, 1974) or with lard (Glennly *et al.*, 1978) increased PAP activities and the rates of hepatic triacylglycerol synthesis. However, the inclusion of sucrose (Glennly *et al.*, 1978; Table 4), beef tallow (Table 4) or mustard-seed oil or corn oil (Kako & Peckett, 1981) did not alter PAP activity. The changes in PAP activity after corticotropin injection were much larger than those for the microsomal and mitochondrial GPAT activities (Table 4). However, the combined effect of these increases probably helps to facilitate the increase in hepatic triacylglycerol synthesis that occurs after the injection of corticotropin or glucocorticoids (Glennly & Brindley, 1978). High-fat diets in themselves seem to have little effect on the basal PAP activities in the liver but they do appear to enhance the corticosterone response to stress or to the consumption of other nutrients (Brindley *et al.*, 1981). The subsequent changes in PAP activity and in triacylglycerol synthesis may therefore be amplified. This is discussed by Brindley *et al.* (1981).

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