The Effects of Diet on the Esterification of Glycerol Phosphate, Dihydroxyacetone Phosphate and 2-Hexadecylglycerol by Homogenates of Rat Adipose Tissue

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1. Male rats were fed for 5 weeks after weaning on a diet containing (by weight) 59% of starch or on diets that contained 39% of starch and 20% of either sucrose, beef tallow or corn oil. 2. The rats fed on the beef tallow consumed more energy than did the rats fed on the starch and sucrose diets. The rats fed on the corn oil drank less water than did the other groups of rats. 3. There were no significant differences between the four groups in terms of body-weight gain, epididymal-fat-pad weight and in the size, number and triacylglycerol content of the adipocytes in the fat-pads. 4. There was a significant correlation ($P < 0.001$) between the activities of glycerol phosphate acyltransferase and monoacylglycerol acyltransferase in individual rats. Both of these activities were highest in the group fed on the high-starch diet and both correlated with the consumption of glucose by individual rats in the four groups. 5. The percentage of glycerol phosphate converted into diacylglycerol and triacylglycerol was positively correlated with the mean diameters, surface area and triacylglycerol content of the adipocytes for individual rats and was greatest in the sucrose-fed rats. 6. The specific activity of dihydroxyacetone phosphate acyltransferase was highest in the rats fed on beef tallow. This activity was positively correlated with the energy intake for all dietary groups over the 5-week feeding period. 7. The results are discussed in terms of the functions of the three routes of glycerolipid synthesis in adipose tissue.

We (Dodds *et al.*, 1976) have demonstrated that glycerolipids can be synthesized in rat adipose tissue from glycerol phosphate, dihydroxyacetone phosphate and from monoacylglycerol. The physiological relationship between these pathways and their relative importance in the synthesis of glycerolipids in adipose tissue is at present unknown. To investigate this problem, rats were fed on diets that differed in their compositions with respect to carbohydrate and to fat. The carbohydrate consisted of starch or sucrose and this enabled the influence of dietary fructose (as sucrose) to be evaluated. The effects of these diets were compared with those obtained with diets that were supplemented with corn oil (a polyunsaturated plant oil) or with beef tallow (a more saturated animal fat). The relative specific activities of the three pathways of glycerolipid synthesis were measured in the fat-pads of rats maintained on these diets.

It is hoped that the results will contribute to the understanding of the relationship between the different pathways of glycerolipid synthesis. In

addition, the results may also indicate how dietary modification may be used to change the rate at which triacylglycerol is synthesized in adipose tissue.

Materials and Methods

Most of the materials have been described in the preceding paper (Dodds *et al.*, 1976). Vitamins A, D and E (Advitamin mixtures) were obtained from Food Industries Ltd., Bromborough, Cheshire, U.K.; water-soluble vitamins and salts were from Hopkin and Williams, Chadwell Heath, Essex, U.K., BDH Chemicals Ltd., Poole, Dorset, U.K., or Koch–Light Laboratories Ltd., Colnbrook, Bucks., U.K. Fats were supplied by Van den Berghs and Jurgens Ltd., Burgess Hill, Sussex, U.K. Casein SLR (low-vitamin content) was purchased from Fisons Scientific Apparatus, Loughborough, Leics., U.K.

Animals and diets

Male weanling rats of the Colworth–Wistar strain were randomly allocated to four different diets and

maintained on these for 5 weeks. The composition (g/100g) of the starch diet was 49% starch, 25% casein, 6% cellulose, 4% salt mixture (Jones & Foster, 1942), 4% vitamin B complex, 4% vitamin B supplement, 1% vitamin A, D and E mixture, 1% of 15% (w/w) L-cystine in starch and 5% ground-nut oil. In the other three diets starch constituted 29g/100g of diet, and 20g/100g was sucrose, beef tallow or corn oil respectively. The vitamin B complex contained 12mg of vitamin B₁₂, 80mg of biotin, 88g of inositol, 40g of *p*-aminobenzoic acid, 6g of folic acid and 15.866kg of starch. The vitamin B supplement was made from 1.83g of thiamin hydrochloride, 2.38g of riboflavin, 1.1g of pyridoxine, 1.88g of ascorbic acid, 11g of pantothenic acid, 0.58g of menaphthone, 220g of nicotinic acid, 1.28kg of choline chloride and 16kg of starch. The vitamin A, D and E mixture was made from 20g of Advitamin A/D₃ (50000/10000 i.u./g), 20g of Advitamin A (50000 i.u./g), 660g of Advitamin E (100 i.u./g) and 9.3kg of starch. The addition of vitamin and salt supplements contributed about 10g per 100g of starch to each of the diets.

Preparation of the fat-free homogenates and the determination of enzyme activities

These methods have been described previously (Dodds *et al.*, 1976). The rates of esterification of glycerol phosphate and hexadecylglycerol were each measured by using four different concen-

trations of homogenate protein, and those for the esterification of dihydroxyacetone phosphate were measured at three concentrations of protein. This ensured that the rates were proportional to the amount of enzyme protein added. The rates were also constant with respect to time for the 30 min incubation periods.

Measurement of the size of adipocytes

The size of adipocytes was determined with fixed frozen sections of adipose tissue by a modification of the microscopic method of Sjöström *et al.* (1971). The volume of each cell was calculated by computer, by assuming that adipocytes are spherical, and the mass of triacylglycerol per cell was estimated by assuming a density of 0.915 g/ml. The triacylglycerol content of adipose tissue was measured by weighing the diethyl ether-extractable lipid, and the number of fat-cells in the fat-pad was estimated by dividing the mass of triacylglycerol per pad by the mass of triacylglycerol per cell (Kirtland *et al.*, 1975).

Determination of the fatty acid composition of triacylglycerols

Triacylglycerol fatty acids were converted into their methyl esters by transmethylation and analysed by g.l.c. (Appleby *et al.*, 1974).

All other determinations are described by Dodds *et al.* (1976).

Table 1. *Food intake of rats fed on the experimental diets*

The results are expressed as means $\pm$ 1 s.d. from six rats in each group. The energy intake is calculated on the assumption that 1g of carbohydrate, protein and fat are equivalent to 15.9, 17.1 and 38.9 kJ respectively (McCance & Widdowson, 1960). The significance of the difference between the four dietary groups (I-IV) is indicated in the Table by: **P* < 0.05; ***P* < 0.02; ****P* < 0.01; †*P* < 0.005; ††*P* < 0.001 for the groups indicated, where v = versus.

Time after weaning	Food consumption (g)				Energy intake (MJ)			
	Starch diet (I)	Sucrose diet (II)	Beef-tallow diet (III)	Corn-oil diet (IV)	Starch diet (I)	Sucrose diet (II)	Beef-tallow diet (III)	Corn-oil diet (IV)
0-7 days	101 $\pm$ 7 I v II†	104 $\pm$ 5 II v III††	86 $\pm$ 4	78 $\pm$ 13 I v IV† II v IV†	1.44 $\pm$ 0.10 I v III**	1.48 $\pm$ 0.07 II v III*	1.59 $\pm$ 0.07	1.44 $\pm$ 0.22
7-14 days	125 $\pm$ 8 I v III††	124 $\pm$ 4 II v III††	107 $\pm$ 4	103 $\pm$ 7 I v IV†† II v IV††	1.78 $\pm$ 0.10 I v III†	1.78 $\pm$ 0.05 II v III†	1.97 $\pm$ 0.07	1.90 $\pm$ 0.12 II v IV*
15-21 days	133 $\pm$ 5 I v III††	128 $\pm$ 18	118 $\pm$ 4	109 $\pm$ 11 I v IV**	1.89 $\pm$ 0.07 I v III††	1.82 $\pm$ 0.25 II v III***	2.18 $\pm$ 0.07	2.01 $\pm$ 0.20
22-28 days	144 $\pm$ 5 I v III††	152 $\pm$ 8 II v III††	125 $\pm$ 7	125 $\pm$ 8 I v IV†† II v IV††	2.05 $\pm$ 0.05 I v II* I v III††	2.17 $\pm$ 0.10	2.31 $\pm$ 0.12	2.30 $\pm$ 0.32
29-35 days	144 $\pm$ 6 I v III††	147 $\pm$ 7 II v III††	120 $\pm$ 5	114 $\pm$ 5 I v IV†† II v IV††	2.06 $\pm$ 0.07 I v III†	2.10 $\pm$ 0.10	2.21 $\pm$ 0.07 III v IV*	2.10 $\pm$ 0.07
0-35 days	646 $\pm$ 28 I v III††	655 $\pm$ 20 II v III††	556 $\pm$ 18	529 $\pm$ 39 I v IV†† II v IV††	9.23 $\pm$ 0.37 I v III††	9.33 $\pm$ 0.27 II v III††	10.25 $\pm$ 0.29	9.75 $\pm$ 0.66

Results and Discussion

Effects of diet on the growth of rats and their consumption of food and water

At the beginning of the feeding period the rats had a mean body weight of 75 ± 4 g (s.d. from 24 rats), and after 5 weeks this had risen to 253 ± 10 g (s.d. from 24 rats). There were no significant differences in the weekly weight gains of the four groups of rats. The weight of food consumed by the rats fed on the diets rich in beef tallow and corn oil was less ($P < 0.05$) than that of the two other groups (Table 1). The rats fed on beef tallow consumed significantly more energy (expressed in terms of MJ) than the rats fed on the starch and sucrose diets. The results indicated that the rats were able to regulate their energy intakes and weight gains on the different diets given in the present studies. It is known that this ability can be over-ridden if the proportion of energy taken in the form of fat exceeds 40% of the total intake. In these circumstances the rats become obese (Lemonnier *et al.*, 1971).

The rats fed on the corn-oil-enriched diet drank about 14% less ($P < 0.02$) water over the 5-week period than did the other groups of rats. This decreased intake was most marked in the first week. The reason for these observations is not known. Baile *et al.* (1973) have claimed that prostaglandins injected into the hypothalamus can alter the consumption of food and water. It is possible that an altered prostaglandin metabolism could result from the high linoleic acid intake in the corn-oil-fed rats and that this may contribute to the decreased water intake in this group.

Effect of diet on the composition of adipose tissue

No significant differences were observed in the size and triacylglycerol content of adipocytes obtained from the four dietary groups (Table 2). There were slight indications that the weight of the fat-pad and the number of cells was higher in the groups fed on the diets enriched with beef tallow and corn oil (Table 2). This parallels the energy consumption of these two groups (Table 1). However, with the number of animals used, the energy consumption was

only higher for the rats fed on beef tallow as compared with the rats fed on the two low-fat diets. In separate experiments (J. Kirtland & M. I. Gurr, unpublished work), it was shown that when the diet was manipulated so that rats increased their energy intake in the first few weeks after weaning, the number of cells in the fat-pads increased. This was not dependent on the composition of the diet.

The fatty acid composition of adipose-tissue triacylglycerol was also measured in the present studies and the major differences were found in the relative proportions (g/100 g of fatty acid) of linoleic acid. In the rats fed on the tallow-rich diet, linoleic acid constituted $6 \pm 0.5\%$ (s.d. from 6 rats) of the fatty acids and was significantly lower ($P < 0.001$) than the values of $8 \pm 1\%$ and $9 \pm 0.5\%$ (s.d. from 6 rats) for the rats fed on the sucrose and starch diets respectively. Linoleic acid accounted for $37 \pm 2\%$ of the fatty acids in the adipose-tissue triacylglycerols of rats fed on the corn-oil diet, and this was significantly higher ($P < 0.001$) than the equivalent results for the three other groups. This is in agreement with the work of Beare & Kates (1964) and showed that the adipose-tissue fatty acid composition reflected that of the diet.

Effect of diet on the activities of the three pathways of glycerolipid synthesis in adipose tissue

The activities of the three pathways of glycerolipid synthesis are shown in Table 3. As observed in the preceding paper (Dodds *et al.*, 1976) the relative rates at which the precursors of glycerolipid synthesis were incorporated into lipids decreased in the order glycerol phosphate > dihydroxyacetone phosphate > 2-hexadecylglycerol. The results have been expressed as specific activities (related to protein) and this seems to be justified, since the protein content of the fat-pads from the rats on the different diets did not differ significantly.

The mean specific activities of glycerol phosphate acyltransferase (EC 2.3.1.15; as measured by the total rate of glycerol phosphate esterification) and monoacylglycerol acyltransferase (EC 2.3.1.22; as measured by the rate of esterification of hexadecyl-

Table 2. *Fat-cell size and number in the epididymal fat-pads*

Results are expressed as means $\pm$ s.d. and there were six rats in each group. There were no significant differences ($P > 0.05$) between the corresponding values for the four groups.

Diet	Wet wt. of fat-pad (g)	Mean cell diameter (μ m)	Mean cell surface area ($\text{cm}^2/10^6$ cells)	Triacylglycerol per cell (μ g)	$10^{-6} \times$ Number of cells in fat-pads
Starch diet	2.91 ± 0.54	93 ± 9	275 ± 48	0.39 ± 0.10	5.7 ± 0.5
Sucrose diet	3.01 ± 0.43	94 ± 8	276 ± 45	0.40 ± 0.10	5.8 ± 0.8
Beef-tallow diet	3.28 ± 0.18	93 ± 5	275 ± 29	0.40 ± 0.06	7.0 ± 1.6
Corn-oil diet	3.38 ± 0.41	96 ± 7	284 ± 41	0.41 ± 0.09	7.1 ± 1.8

Table 3. *Effect of diet on the activities of the three pathways of glycerolipid biosynthesis in rat adipose tissue*

Six rats were allocated to each dietary group (see Table 1) for 5 weeks and then the rates of glycerolipid synthesis were measured in the fat-free homogenate of the epididymal fat-pad (see the Materials and Methods section). The activity of the glycerol phosphate pathway is expressed as nmol of glycerol phosphate incorporated into lipids/min per mg of homogenate protein and that of the two other pathways as nmol of palmitate esterified/min per mg of homogenate protein. Results are given as means $\pm$ 1 s.d. Significant differences are indicated as in Table 1.

Dietary group	Rate of esterification of			
	Glycerol phosphate into		Dihydroxyacetone phosphate	Hexadecylglycerol
	Total lipid	Neutral lipid		
I Starch diet	16.5 $\pm$ 2.6 I v II††	3.5 $\pm$ 1.3 I v III†	1.50 $\pm$ 0.14 I v III††	0.87 $\pm$ 0.13 I v II*
II Sucrose diet	9.5 $\pm$ 2.2	3.4 $\pm$ 0.8 II v IV††	1.46 $\pm$ 0.27	0.60 $\pm$ 0.13
III Beef-tallow diet	8.4 $\pm$ 1.7 I v III††	1.1 $\pm$ 0.2 II v III††	1.97 $\pm$ 0.16 II v III***	0.58 $\pm$ 0.09 I v III*
IV Corn-oildiet	8.7 $\pm$ 2.0 I v IV††	1.5 $\pm$ 0.4 I v IV**	1.52 $\pm$ 0.31 III v IV*	0.65 $\pm$ 0.12

Table 4. *Composition of the products obtained from the esterification of sn -[1,3- 3 H]glycerol 3-phosphate*

The compositions of the products obtained in the assays of glycerol phosphate esterification (Table 3) are expressed as means $\pm$ 1 s.d. The significance of the difference between the lipids synthesized by the fat-free homogenates of adipose tissue obtained from the rats fed on the sucrose diet and those synthesized by tissue from the other groups is indicated in the Table. There were six rats in each group.

Dietary group	Composition of products (mol/100mol)				
	Lysophosphatidate	Phosphatidate	Diacylglycerol	Triacylglycerol	Diacylglycerol plus triacylglycerol
Starch diet	19 $\pm$ 5	60 $\pm$ 9 $P < 0.05$	7 $\pm$ 3	14 $\pm$ 5 $P < 0.001$	21 $\pm$ 8 $P < 0.005$
Sucrose diet	18 $\pm$ 9	46 $\pm$ 9	9 $\pm$ 3	27 $\pm$ 4	36 $\pm$ 3
Beef-tallow diet	18 $\pm$ 8	61 $\pm$ 11 $P < 0.05$	6 $\pm$ 4	15 $\pm$ 6 $P < 0.005$	21 $\pm$ 9 $P < 0.005$
Corn-oil diet	21 $\pm$ 9	63 $\pm$ 13 $P < 0.05$	6 $\pm$ 2	10 $\pm$ 5 $P < 0.001$	16 $\pm$ 7 $P < 0.001$

glycerol) were higher in the rats fed on the starch diet than in the other groups (Table 3). All of these differences were significant except when the rates of hexadecylglycerol esterification were compared in the rats fed on the starch and corn-oil diets. In the latter case $0.1 > P > 0.05$.

The major product of glycerol phosphate esterification for all four diets was phosphatidate (Table 4). However, this proportion was lower for the rats fed on the sucrose diet and correspondingly more glycerol phosphate was converted into triacylglycerol. The homogenates prepared from the rats fed on the starch and sucrose diets catalysed approximately twice the molar flux through phosphatidate phosphohydrolase (EC 3.1.3.4) than did those from the rats given corn oil and beef tallow (Table 3). These results are compatible with an increased activity of phosphatidate phosphohydrolase and of diacylglycerol

acyltransferase (EC 2.3.1.20). However, direct determinations of the activities of these enzymes were not performed and the assay systems used may not have been optimum for these activities. Lamb & Fallon (1974) and Fallon *et al.* (1975) have already concluded that feeding diets rich in glucose and fructose to rats increases the activity of phosphatidate phosphohydrolase and diacylglycerol acyltransferase in the liver.

There were significant correlations ($r > 0.52$; $P < 0.05$) between the mean diameters, the surface areas and the triacylglycerol content of adipocytes of individual rats fed on the starch, beef-tallow and corn-oil diets (Table 2) and the percentage of sn -[1,3- 3 H]glycerol 3-phosphate recovered in the neutral lipid fractions (Table 4). In this respect the sucrose-fed rats clearly belonged to a different population (Table 4). However, significant corre-

lations ($r > 0.82$; $P < 0.05$) were again found between the percentage of the glycerol phosphate isolated as neutral lipid and the various parameters of cell size when the results from these six rats were analysed separately. These findings agree with other observations that larger adipocytes have a greater capacity for triacylglycerol synthesis (Zinder *et al.*, 1967; Smith, 1971; Holm *et al.*, 1975).

The relationship between the rates of esterification of the three precursors of glycerolipid synthesis was determined by subjecting the individual results, which were summarized in Table 3, to linear-regression analysis and determining the product moment correlation coefficient. There was a highly significant correlation between the rates of esterification of glycerol phosphate and of hexadecylglycerol ($r = 0.81$; $P < 0.001$). By contrast, the rate of dihydroxyacetone phosphate esterification did not correlate with either the rate of esterification of glycerol phosphate ($r = -0.1$) or hexadecylglycerol ($r = -0.2$). These results therefore confirm that glycerol phosphate acyltransferase and dihydroxyacetone phosphate acyltransferase are distinct enzymes. Moreover, they appear to have different physiological functions, since dietary modification affects their activities in completely different ways.

The conversion of carbon atoms from glucose into the glycerol backbone of glycerolipids via glycerol phosphate requires NADH in the glycerol phosphate dehydrogenase reaction (EC 1.1.1.8). However, direct incorporation via dihydroxyacetone phosphate involves NADPH in the reduction of acyldihydroxyacetone phosphate (LaBelle & Hajra, 1972). NADPH is normally a cofactor for reductive synthesis, whereas NADH is involved in degradative pathways. This observation has been used as an argument for the importance of the dihydroxyacetone phosphate pathway of glycerolipid synthesis (Harding *et al.*, 1975; Pollock *et al.*, 1975).

It might therefore be expected that the activity of the dihydroxyacetone phosphate acyltransferase would be high in conditions of elevated fatty acid synthesis and NADPH production. This should have occurred with the rats fed on the high-starch diet. Addition of sucrose (and particularly fructose) to the diet decreases fatty acid biosynthesis in rat adipose tissue and appears to shift the site of fatty acid production to the liver (Bruckdorfer *et al.*, 1971; Chevalier *et al.*, 1972). Increasing the proportion of fat in the diet decreases the rate of fatty acid synthesis in the adipose tissue and livers of rats, pigs and chickens (Allee *et al.*, 1971; Romsos & Leveille, 1974; Harding *et al.*, 1975). Feeding tallow decreased fatty acid biosynthesis in adipose tissue more than did safflower oil, whereas the opposite effect was found in the liver of rats (Waterman *et al.*, 1975). In addition acetyl-CoA carboxylase activities were higher in the adipose

tissue of rats fed on diets rich in linoleic acid rather than oleic acid (Loriette & Raulin, 1972). By contrast, the activity of dihydroxyacetone phosphate acyltransferase was highest in the rats fed on the beef-tallow diet (Table 3) and so the activity of this enzyme does not appear to parallel the rate of fatty acid biosynthesis. Linear-regression analysis of the results for individual rats from the four groups showed a significant positive correlation ($r = 0.62$; $P < 0.01$) between dihydroxyacetone phosphate acyltransferase activity and the energy intake over the 5-week period of feeding (Table 1). This may indicate that the enhanced acyltransferase activity does not result from a specific effect of the tallow diet.

The activities of both glycerol phosphate acyltransferase and monoacylglycerol acyltransferase were highest in the starch diet, in which the rates of fatty acid biosynthesis in adipose tissue should have been increased. A close parallel between the activities of glycerol phosphate acyltransferase and fatty acid synthetase was observed in the developing mammary gland of rabbits (Short *et al.*, 1977; D. N. Brindley & R. Dils, unpublished work), but this relationship was not found in rat liver (Wiegand *et al.*, 1974).

The increased activity of glycerol phosphate acyltransferase and monoacylglycerol acyltransferase observed in the rats fed on the high-starch diet (Table 3) may have been partly caused by the effect of insulin. Insulin has been shown to stimulate the adipose-tissue activities of acyl-CoA synthetase (EC 6.2.1.3; Jason *et al.*, 1976) and glycerol phosphate acyltransferase (Sooranna & Saggerson, 1976). However, their observations probably result from an acute regulation of metabolism, whereas in the present work chronic adaptations would also have taken place. Smith *et al.* (1974) demonstrated that glucose feeding renders fat-cells more sensitive to insulin. In the present work there were significant correlations between the glucose consumed (as starch and sucrose) by the individual rats and the activities of glycerol phosphate acyltransferase ($r = 0.76$; $P < 0.001$) and monoacylglycerol acyltransferase ($r = 0.48$; $P < 0.05$).

The physiological function of monoacylglycerol acyltransferase in adipose tissue is not known. If it were to be responsible for the esterification of monoacylglycerols which are absorbed from the blood, then it should have been increased in conditions of enhanced flux of triacylglycerol from the liver and intestine to the adipose tissue. This should have occurred on the sucrose diet (Bruckdorfer *et al.*, 1972) as well as on the beef-tallow and corn-oil diets. An alternative hypothesis is that the monoacylglycerol acyltransferase is concerned with the recycling of monoacylglycerols, derived from lipolysis of the fat stores, back to triacylglycerols.

This activity closely parallels the rate of glycerolipid synthesis *de novo* from glycerol phosphate.

The changes in enzyme activities reported in Table 3 were not accompanied by a significant alteration in triacylglycerol accumulation in the epididymal fat-pads (Table 2). This may mean that the turnover of triacylglycerol is increased in conditions where the activity of one of the acyl-transferases is elevated. Alternatively, the specific activity as measured *in vitro* could represent a capacity for synthesis that may not be expressed *in vivo*. The results from the present experiments demonstrate the effects of diet on three separate routes of glycerolipid synthesis in adipose tissue. The physiological significance of each route of synthesis and their interrelationship is still obscure. Nevertheless, all these pathways should be taken into account when discussing adipose-tissue metabolism.

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