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The Effect of Dietary Fat on Lipogenesis in Mammary Gland and Liver from Lactating and Virgin Mice

By STUART SMITH, HARRIET T. GAGNÉ, DOROTHY R. PITELKA AND S. ABRAHAM

The Bruce Lyon Memorial Research Laboratory, Children's Hospital Medical Center of Northern California, Oakland, Calif. 94609, *U.S.A., and Department of Zoology and its Cancer Research Genetics Laboratory, University of California, Berkeley, Calif.* 94720, *U.S.A.*

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1. Virgin and lactating C_3H mice maintained on laboratory chow were transferred to a high-fat (15% corn oil) or a fat-free diet 3 days before being killed. 2. The linoleate content of liver, mammary gland and milk was decreased in lactating mice given the fat-free diet but was increased in those fed on the high-fat diet. Changes in linoleate content and mammary gland followed a similar but much less marked trend in virgin animals. 3. Hepatic fatty acid synthesis in lactating and virgin mice fed on the fat-free diet was higher than in corresponding animals fed on either the chow or the high-fat diet. The lipogenic capacity of livers from mice fed on either the chow or the high-fat diet was greater in lactating than in virgin animals. These changes in hepatic lipogenic capacity were accompanied by alterations in the specific activities of certain enzymes involved in fat synthesis. 4. Mammary gland from virgin and lactating animals showed no such adaptation to dietary fat. Results indicate that fatty acid synthesis in neither mammary-gland parenchymal cells nor mammary-gland adipose cells can be influenced by dietary fat in the same way as in the hepatocyte.

Lipogenesis in liver and adipose tissue of non-lactating animals is generally believed to be profoundly influenced by nutritional status (Masoro, Chaikoff, Chernick & Felts, 1950; Whitney & Roberts, 1955; Hausberger & Milstein, 1955). The feeding of diets high in carbohydrate and low in fat stimulates lipogenesis, whereas starving or feeding of diets rich in fat leads to decreased lipogenesis. These gross metabolic changes appear to be closely related to the adaptive behaviour of certain enzymes (Pitot, Peraino, Pries & Kennan, 1964; Ball, 1966; Leveille & Hanson, 1966; Goodridge, 1968).

Little information is available on the response of the lactating animal to dietary manipulations. Coniglio & Culp (1965) have reported that mammary glands from lactating rats that had been starved for 48hr. showed decreased lipogenesis, and they suggested that this tissue too might be subject to the same kind of dietary control known to function in the liver and adipose tissue of non-lactating animals. However, the interpretation of such findings may be complicated by the fact that the mammary gland consists of a mixed population of cell types, the lactating gland being composed mainly of parenchymal cells and virgin gland mainly of adipose cells.

The activities of a number of hepatic enzymes concerned with lipogenesis have been shown to be higher in lactating than in virgin animals, but it has not been shown whether these increased enzyme activities are accompanied by an increase in hepatic lipogenic capacity (McLean, 1958; Willmer, 1960; Bartley, Abraham & Chaikoff, 1966). On the contrary, Dannenburg, Burt & Leake (1964) found that hepatic lipogenesis in puerperal rats was not higher than in non-pregnant animals.

We have therefore compared the effect of fat-free and high-fat diets on lipogenesis in the liver and mammary gland of virgin and lactating mice. In order to correlate any changes in lipogenic capacity with changes in enzyme activity, rates of fatty acid synthesis in tissue slices from various labelled substrates have been measured in conjunction with the activities of certain soluble enzymes concerned with lipogenesis. Results obtained with mice fed on a chow diet are included as this was the diet given to the animals before the test diets. The results therefore give an indication of the metabolic state of the mice before they received test diets.

EXPERIMENTAL

Materials. All ^{14}C- and ^{3}H-labelled compounds were purchased from New England Nuclear Corp., Boston, Mass., U.S.A. Substrates, cofactors and auxiliary enzymes

Table 1. *Composition of diets*

Gross analysis

Component	Proportion in diet (g./100g. of diet) Fat-free diet	High-fat diet
Glucose	50	50
Casein, vitamin-free	22	22
Salt mixture*	6	6
Defatted liver†	2	2
Vitamin B mixture‡	1	1
Cellulose	19	4
Corn oil	0	15

Lipid analysis

Fatty acid	Proportion in diet (moles/100 moles of fatty acid) Chow diet	High-fat diet
$C_{14:0}$	1·6	0
$C_{16:0}$	20·3	11·6
$C_{16:1}$	2·0	0
$C_{18:0}$	10·8	1·9
$C_{18:1}$	31·2	21·9
$C_{18:2}$	27·9	61·4
$C_{18:3}$	1·3	1·2
$>C_{18}$	0	0
g. of fat/100g. of diet ...	11	15

* Salt Mixture U.S.P. XIV (Nutritional Biochemicals Corp., Cleveland, Ohio, U.S.A.).

† Jayron Powder (VioBin Corp., Monticello, Ill., U.S.A.).

‡ Hill & Chaikoff (1954).

required in the enzyme assays were purchased from California Corp. for Biochemical Research, Los Angeles, Calif., U.S.A., Sigma Chemical Co., St Louis, Mo., U.S.A., and C. F. Boehringer und Soehne G.m.b.H., Mannheim, Germany, and were of the highest purity available.

Animals. C_3H mice were given Purina mouse chow and water *ad libitum* unless otherwise stated. Lactating mice (10–17 days *post partum*, suckling at least five pups) or virgin mice (2½–3 months old) were fed on the special diets for 3 days before being killed. The compositions of the various diets are shown in Table 1. No attempt was made to keep the caloric content per unit weight of diet constant, for Hill, Linazasoro, Chevallier & Chaikoff (1958) have shown that the caloric value of the diet does not affect the response of hepatic lipogenesis to changes in dietary fat content. Pups continued to gain weight when dams were transferred to the test diets, and the dams themselves showed no greater loss in weight than is normal during the lactational period. All animals were killed by cervical fracture. Food was present in the stomachs of all animals. Tissue slices (Abraham, Madsen & Chaikoff, 1964) and homogenate fractions (Abraham, Kopelovich, Kerkof & Chaikoff, 1965) were prepared as described previously.

Milk collection and analysis. Groups of lactating mice maintained on the various diets were kept separate for the specific purpose of collection and analysis of milk. After feeding the lactating mice with the diets for 3 days, we gave the animals a subcutaneous injection of Pitocin (1 unit), and milked them 15min. later by applying gentle suction to the nipples.

Lipids were extracted from milk with chloroform–methanol (2:1, v/v) (20vol.) and the extract was washed by the procedure of Folch, Lees & Sloane-Stanley (1957). After weighing the dried lipid extracts, we carried out methanolysis by the method of Luddy, Barford & Riemenschneider (1960).

Analysis of tissue lipids. Tissue lipids were extracted with chloroform–methanol (2:1, v/v), saponified and methylated with diazomethane (Arndt, 1943).

Incubation of tissue slices. Portions (100mg.) of mammary-gland slices (0·4mm. thick) were incubated in 1·0ml. of sodium bicarbonate buffer, pH7·4 (Krebs & Henseleit, 1932), containing the following ^{14}C- or ^{3}H-labelled and unlabelled substrates: labelled glucose (10μmoles) with unlabelled acetate (10μmoles); labelled acetate (10μmoles) with or without unlabelled glucose (10μmoles); labelled L-leucine (2μmoles) with unlabelled glucose (10μmoles).

Portions (200mg.) of liver slices (0·4mm. thick) were incubated in 2·0ml. of the Krebs–Henseleit bicarbonate buffer containing either labelled acetate (4μmoles) or labelled pyruvate (10μmoles).

After incubating liver or mammary-gland slices at 37° for 3hr. under $O_2 + CO_2$ (95:5), we determined the radiochemical yields of CO_2 (Bartley & Abraham, 1966) and fatty acids (Abraham, Matthes & Chaikoff, 1961) with the Ansitron Liquid Scintillation Spectrometer as described previously.

Conversion of labelled substrates into CO_2 and fatty acids was directly proportional to time and to the amount of tissue present over the 3hr. incubation period.

Enzyme assays. All enzyme assays were performed at 30° on the $6 \times 10^6 g$-min. supernatant fraction obtained from tissue homogenates. Except for the acetyl-CoA carboxylase reaction, all assays were carried out spectrophotometrically by observing the change in extinction at 340nm. All reaction mixtures were preincubated for 5min. at 30° before addition of substrate. Reaction rates were proportional to enzyme concentration and time of incubation. Blank values, obtained by omitting substrate from the medium, were always determined. The extinction coefficient of $6{\cdot}22 \times 10^6$cm.^{2}/mole at 340nm. was used (Horecker & Kornberg, 1948) and specific activities are expressed in nmoles of nicotinamide nucleotide oxidized or reduced/min./mg. of protein, unless otherwise stated.

Hexokinase (ATP–D-hexose 6-phosphotransferase, EC 2.7.1.1) and glucokinase (ATP–D-glucose 6-phosphotransferase, EC 2.7.1.2) were assayed by a modification of the method of Viñuela, Salas & Sols (1963). Assay systems contained in a final volume of 2·0ml.: histidine hydrochloride–tris (100μmoles) adjusted to pH8·0 with KOH, EDTA (10μmoles), $MgCl_2$ (20μmoles), $NADP^+$ (0·62μmole), ATP (3·0μmoles) and non-rate-limiting amounts of glucose 6-phosphate dehydrogenase (D-glucose 6-phosphate–NADP oxidoreductase, EC 1.1.1.49) and 6-phosphogluconate dehydrogenase [6-phospho-D-gluconate–NADP oxidoreductase (decarboxylating), EC 1.1.1.44]. Blank values, obtained by omitting ATP from the medium, were subtracted from each determination. For liver preparations, reactions were carried out with either glucose (1μmole or 200μmoles) or *N*-acetylglucosamine (100μmoles), and glucokinase activity was assayed by the method of Viñuela *et al.* (1963). Mammary-gland preparations were assayed

at the single glucose content of 200 μmoles since this tissue does not contain glucokinase activity. The amount of glucose phosphorylated was then calculated by dividing by 2 the amount of NADPH produced. Specific activities of glucokinase (from liver) and hexokinase (from mammary gland) are thus expressed as nmoles of glucose phosphorylated/min./mg. of protein.

'Malic' enzyme [L-malate–NADP oxidoreductase (decarboxylating), EC 1.1.1.40] was assayed by a modification of the method of Ochoa (1955). Assay systems contained glycylglycine–KOH buffer pH 7·5 (75 μmoles), $NADP^+$ (0·25 μmole) and L-malate (2 μmoles) in a final volume of 2·0 ml.

Citrate-cleavage enzyme [ATP–citrate oxaloacetate-lyase (CoA-acetylating and ATP-dephosphorylating), EC 4.1.3.8] was determined by the spectrophotometric method of Srere (1959).

Fatty acid synthetase assay systems contained potassium phosphate buffer, pH 6·6 (50 μmoles), dithiothreitol (0·5 μmole), acetyl-CoA (25 nmoles), NADPH (75 nmoles) and malonyl-CoA (27 nmoles) in a final volume of 0·5 ml.

Acetyl-CoA carboxylase [acetyl-CoA–CO_2 ligase (ADP), EC 6.4.1.2] assay systems contained potassium phosphate buffer pH 7·2 (100 μmoles), dithiothreitol (1 μmole), potassium citrate (20 μmoles), $MgCl_2$ (10 μmoles), $MnCl_2$ (0·5 μmole), ATP (3 μmoles), defatted human serum albumin (1·0 mg.) and $KH^{14}CO_3$ (10 μmoles, 2 μc) in a final volume of 1·0 ml. After assay mixtures had been incubated for 10 min. at 30°, reactions were started by the addition of 150 nmoles of acetyl-CoA. The amount of [^{14}C]malonyl-CoA produced was determined by the method of Smith, Easter & Dils (1966). Specific activities are expressed in nmoles of bicarbonate converted into malonyl-CoA/min./mg. of protein.

Glucose 6-phosphate dehydrogenase was assayed by the method of Horecker & Smyrniotis (1955) except that a non-rate-limiting amount of 6-phosphogluconate dehydrogenase was added to the system. The amount of NADPH produced was then divided by 2 to give the amount of glucose 6-phosphate utilized.

α-Glycerol phosphate dehydrogenase (L-glycerol 3-phosphate–NAD oxidoreductase, EC 1.1.1.8) was assayed according to Beisenhertz, Bucher & Garbade (1955) except that 75 mM glycylglycine–KOH, pH 7·5, replaced triethanolamine–HCl as buffer.

Proteins were determined by the biuret method of Gornall, Bardawill & David (1949), with defatted human serum albumin as standard.

Gas–liquid chromatography. Radioactive fatty acids from tissue-slice incubations were converted into their methyl esters with an excess of diazomethane and fractionated on a Warner–Chilcott gas chromatograph. A stainless-steel column (6 ft. × ½ in.) containing 15% diethylene glycol succinate on Chromosorb W (80–100 mesh) was used, with a column flow rate of 60 ml. of argon/min. Temperature was programmed from 100° to 175° at the rate of 50°/min. Methyl esters determined with an ionization detector (10 mc, ^{90}Sr) were trapped in scintillation fluid (10 ml. of toluene containing 50 mg. of 2,4-diphenyloxazole) as they emerged from the column. Recovery of ^{14}C radioactivity from the column was between 80% and 95%.

Methyl esters of milk fatty acids were analysed on a Beckman GC-2A gas chromatograph. A copper column (8 ft. × ½ in.) containing 15% diethylene glycol succinate on Chromosorb W (80–100 mesh) was used isothermally at 203° with a helium flow rate of 96 ml./min. Methyl esters were located in the column effluent with a thermal-conductivity detector. Peak sizes were corrected for variation in detector response to the different methyl esters, by reference to a standard mixture (Wiese, Coon, Yamanaka, Barber & Johnson, 1967).

RESULTS

Hepatic lipogenesis

Lactating animals. Liver slices from lactating mice fed on the fat-free diet incorporated the ^{14}C of [1-^{14}C]acetate and [2-^{14}C]acetate into fatty acids at a higher rate than liver slices from lactating animals fed on a regular chow diet (Table 2). The activities of most of the adaptive enzymes, namely citrate-cleavage enzyme, fatty acid synthetase, glucose 6-phosphate dehydrogenase and glucokinase, were higher in the group fed on the fat-free diet than in the group fed on the chow diet (Table 3). 'Malic' enzyme activity, however, was identical in each group, and acetyl-CoA carboxylase activity was only slightly higher in the group fed on the fat-free diet (Table 3).

Lactating mice fed on the fat-free diet also showed rates of hepatic fatty acid synthesis from acetate carbon that were considerably higher than those from lactating animals fed on the high-fat diet (Table 2). Activities of citrate-cleavage enzyme, acetyl-CoA carboxylase and glucose 6-phosphate

Table 2. *Effect of diet on the metabolism of liver slices from virgin and lactating mice*

Results are expressed as the percentage of added radioactivity converted into fatty acids or CO_2/200 mg. of slices in 3 hr. Results are given as means ± S.E.M., with the numbers of observations in parentheses.

		Chow diet		Fat-free diet		High-fat diet	
Status	Substrate	Fatty acids	CO_2	Fatty acids	CO_2	Fatty acids	CO_2
Virgin	[1-^{14}C]Acetate	1·4 ±0·7 (4)	8·6±1·2 (4)	6·8±2·0 (7)	14·8±0·9 (7)	1·5 ±0·2 (6)	14·0±1·2 (6)
	[1-^{14}C]Pyruvate		52·9±1·4 (4)		71·3±5·1 (7)		64·9±6·3 (6)
	[2-^{14}C]Pyruvate	0·25±0·06 (4)	15·0±1·0 (4)	3·6±0·6 (7)	21·0±1·1 (7)	0·68±0·3 (6)	16·4±1·3 (6)
Lactating	[1-^{14}C]Acetate	2·9 ±0·2 (4)	22·5±1·8 (4)	6·8±1·2 (2)	12·0±0·4 (3)	3·4 ±0·6 (4)	17·0±2·1 (3)
	[2-^{14}C]Acetate	2·9 ±0·3 (4)	9·3±0·9 (4)	7·2±2·7 (2)	10·0±1·0 (3)	3·1 ±0·6 (4)	9·1±1·0 (3)

Table 3. *Effect of diet on enzyme activities from the livers of virgin and lactating mice*

Units of enzyme activity are given in the Experimental section. Results are given as means ± S.E.M., with the numbers of observations in parentheses.

Enzyme	Chow diet		Fat-free diet		High-fat diet	
	Virgin	Lactating	Virgin	Lactating	Virgin	Lactating
Citrate-cleavage enzyme	6·0 ± 1·2 (4)	19·1 ± 1·7 (9)	22·0 ± 2·7 (4)	48·6 ± 4·2 (6)	8·0 ± 0·4 (4)	23·1 ± 3·9 (6)
Acetyl-CoA carboxylase	0·3 ± 0·06 (4)	1·5 ± 0·3 (4)	1·0 ± 0·07 (4)	2·1 ± 0·3 (4)	0·19 ± 0·02 (4)	0·9 ± 0·1 (2)
Fatty acid synthetase	12·0 ± 0·6 (4)	26·4 ± 2·6 (5)	38·0 ± 3·9 (4)	64·0 ± 10 (2)	12·0 ± 0·5 (4)	43·0 ± 8·0 (5)
'Malic' enzyme	24·0 ± 1·7 (4)	55·0 ± 8·9 (9)	41·0 ± 3·7 (4)	57·6 ± 11 (7)	23·0 ± 1·1 (4)	51·5 ± 3·8 (6)
Glucose 6-phosphate dehydrogenase	10·0 ± 1·2 (4)	22·0 ± 5·3 (7)	56·0 ± 4·8 (4)	106 ± 18 (4)	15·0 ± 2·0 (4)	31·0 ± 6·3 (6)
Glucokinase	18·0 ± 2·0 (4)	47·0 ± 4·7 (4)	31·0 ± 1·7 (4)	72·0 ± 11 (4)	24·0 ± 2·0 (4)	58·0 ± 6·3 (4)
α-Glycerol phosphate dehydrogenase	309 ± 10 (4)	201 ± 34 (7)	216 ± 5·0 (4)	138 ± 10 (7)	267 ± 7·0 (4)	177 ± 22 (4)

dehydrogenase were also higher in the group fed on the fat-free diet than in the group fed on the high-fat diet, but there were no significant differences in the activities of fatty acid synthetase, 'malic' enzyme and glucokinase (Table 3).

Virgin animals. Conversion of [1-^{14}C]acetate and [2-^{14}C]pyruvate into fatty acids by liver slices from virgin mice was significantly higher in the group fed on the fat-free diet than in the groups fed on the high-fat or the regular chow diet (Table 2). Activities of citrate-cleavage enzymes, acetyl-CoA carboxylase, fatty acid synthetase, 'malic' enzyme, glucose 6-phosphate dehydrogenase and glucokinase were all higher in the group fed on the fat-free diet than in the groups fed on the chow or the high-fat diet (Table 3).

In virgin mice, under all dietary conditions, the ratio (^{14}C as CO_2)/(^{14}C as fatty acid) was significantly higher with [2-^{14}C]pyruvate as substrate than with [1-^{14}C]acetate as substrate. Thus it was 60·0 for [2-^{14}C]pyruvate and 6·1 for [1-^{14}C]acetate in mice fed on the chow diet, 5·8 for [2-^{14}C]pyruvate and 2·2 for [1-^{14}C]acetate in mice fed on a fat-free diet, and 24·0 for [2-^{14}C]pyruvate and 9·3 for [1-^{14}C]-acetate in mice fed on a high-fat diet. The different values for this ratio may well result from the different intracellular sites of the enzymes producing acetyl-CoA from each of the two substrates: the pyruvate dehydrogenase complex is located in the mitochondrial membrane whereas the acetate–CoA ligase is found predominantly in the soluble portion of the cytoplasm. Thus acetyl-CoA is formed from pyruvate at a subcellular site that favour energy production via the citric acid cycles whereas acetyl-CoA is formed from acetate at a site favouring energy storage as fat.

The increased rate of lipogenesis in animals fed on the fat-free diet is also reflected in these ratios, by the relatively larger proportion of the acetyl-CoA diverted into fat from both substrates. In the animals fed on the fat-free diet the ratios for acetate and pyruvate were much closer to each other than in animals fed on diets high in fat (either chow or high fat). It would seem therefore that transport of acetyl groups from the mitochondria occurs more rapidly under conditions favouring high rates of lipogenesis. In line with this suggestion is the observation that, under these conditions, citrate-cleavage enzyme is high in activity and could therefore facilitate a more rapid transfer of acetyl-CoA from the intra- to the extra-mitochondrial space.

Rates of hepatic fatty acid synthesis from acetate, and also the activities of the enzymes concerned with lipogenesis, were generally higher in lactating mice than in virgin mice. This is in agreement with the results of Bartley *et al.* (1966), who found increased activities of glucose 6-phosphate dehydrogenase, 'malic' enzyme and citrate-cleavage enzyme in livers from lactating animals.

Mammary lipogenesis

Lactating animals. Results obtained with the mammary gland were quite different from those obtained with the liver. The rates of fatty acid synthesis in mammary-gland slices from each of the three groups of animals were almost identical (Table 4). In addition, oxidation of glucose, acetate and leucine was not significantly different in any of the three groups of animals. The only enzyme from lactating mammary gland to show any significant dietary adaptation was citrate-cleavage enzyme (Table 5). The activity of this enzyme was much higher in the lactating mammary gland from animals fed on the fat-free diet than in that from animals fed on either the chow or the high-fat diet.

Virgin animals. In contrast with the mammary glands from lactating animals, mammary glands from virgin animals did respond to dietary changes. Mammary-gland slices from virgin mice fed on either the fat-free or the high-fat diet showed higher rates of lipogenesis than did slices from animals fed on the chow diet when either glucose or acetate was used as substrate (Table 4).

Citrate-cleavage enzyme, acetyl-CoA carboxylase,

Table 4. *Effect of diet on the metabolism of mammary-gland slices from virgin and lactating mice*

Results are expressed as percentage of added radioactivity converted into fatty acids or CO_2/100mg. mammary gland slice in 3hr. Results are given as means ±S.E.M., with the numbers of observations in parentheses.

	Substrate		Chow diet		Fat-free diet		High-fat diet	
Status	Labelled	Unlabelled	Fatty acids	CO_2	Fatty acids	CO_2	Fatty acids	CO_2
Virgin	[1-^{14}C]Glucose	Acetate	1·6 ±0·4 (5)	3·7±0·7 (5)	3·5 ±0·6 (10)	6·7±1·2 (10)	4·1±0·5 (9)	7·8±0·8 (9)
	[6-^{14}C]Glucose	Acetate	2·5 ±0·8 (5)	1·1±0·1 (5)	6·2 ±0·7 (10)	1·8±0·2 (10)	9·0±1·2 (9)	2·0±0·1 (9)
	[2-^{14}C]Acetate	None	0·16±0·06 (2)	1·3±0·2 (2)	0·39±0·07 (5)	1·8±0·3 (5)	0·5±0·07 (2)	2·5±0·3 (4)
	[2-^{14}C]Acetate	Glucose	1·8 ±0·3 (5)	0·8±0·2 (5)	5·2 ±0·8 (10)	1·6±0·2 (10)	6·0±0·47 (9)	1·8±0·2 (9)
	[U-^{14}C]Leucine	Glucose	0·24±0·1 (3)	2·9±0·4 (3)	4·6 ±1·0 (5)	2·9±0·6 (7)	5·2±0·6 (5)	4·3±0·4 (7)
Lactating	[1-^{14}C]Glucose	Acetate	10·7 ±1·4 (7)	22·7±3·2 (7)	12·5 ±1·8 (7)	19·0±2·4 (7)	10·4±1·0 (9)	22·0±2·3 (5)
	[1-^{3}H]Glucose	Acetate	10·7 ±1·3 (7)		8·7 ±1·8 (7)		7·6±0·8 (9)	
	[6-^{14}C]Glucose	Acetate	19·1 ±2·7 (7)	6·9±0·9 (7)	19·4 ±4·3 (7)	6·3±1·2 (7)	16·1±1·4 (9)	4·7±0·8 (5)
	[6-^{3}H]Glucose	Acetate	4·5 ±0·6 (7)		6·7 ±1·0 (7)		6·0±0·6 (9)	
	[2-^{14}C]Acetate	None	0·8 ±0·2 (7)	7·7±1·2 (7)	1·1 ±0·2 (7)	10·8±3·6 (7)	1·2±0·2 (7)	10·8±2·1 (7)
	[2-^{14}C]Acetate	Glucose	29·3 ±2·4 (7)	7·2±1·0 (7)	24·6 ±5·0 (7)	6·7±2·2 (7)	26·4±2·4 (8)	6·6±1·7 (9)
	[2-^{3}H]Acetate	Glucose	6·5 ±0·4 (7)		5·6 ±1·1 (7)		6·0±0·6 (9)	
	[U-^{14}C]Leucine	Glucose	19·8 ±2·3 (7)	9·1±0·9 (6)	17·3 ±1·4 (4)	9·1±2·0 (7)	16·9±1·3 (8)	9·6±1·1 (9)

Table 5. *Effect of diet on the activities of enzymes from the mammary glands of virgin and lactating mice*

Units of enzyme activity are given in the Experimental section. Results are given as means ±S.E.M., with the numbers of observations in parentheses.

	Chow diet		Fat-free diet		High-fat diet	
Enzyme	Virgin	Lactating	Virgin	Lactating	Virgin	Lactating
Citrate-cleavage enzyme	7·0 ± 0·5 (5)	53·3± 9·7 (10)	12·6± 2·9 (10)	100 ±11 (7)	11·0± 1·0 (10)	55·0± 5·2 (6)
Acetyl-CoA carboxylase	0·29 ± 0·06 (5)	8·4± 1·2 (8)	1·5± 0·2 (7)	5·7± 1·0 (4)	1·4± 0·1 (7)	8·8± 0·2 (2)
Fatty acid synthetase	24·0 ± 3·7 (5)	101 ±10 (11)	60·7± 4·8 (10)	94·0± 2·0 (2)	46·6± 4·3 (10)	162 ±20 (5)
'Malic' enzyme	34·0 ± 5·1 (5)	56·0± 9·5 (11)	74·4± 4·5 (10)	57·0±12 (7)	60·5± 5·5 (10)	55·0± 5·1 (6)
Glucose 6-phosphate dehydrogenase	80·0 ± 8·4 (5)	149 ± 8·0 (7)	109 ± 9·0 (5)	152 ± 8·0 (4)	88·0± 5·3 (5)	163 ±13 (6)
Hexokinase	28·0 ± 7·0 (5)	60·0± 7·4 (4)	36·0± 1·9 (7)	65·0± 5·0 (4)	31·0± 1·7 (7)	65·7± 5·3 (4)
α-Glycerol phosphate dehydrogenase	666 ±63 (2)	228 ±21 (9)	613 ±42 (2)	215 ±50 (7)	684 ±50 (2)	265 ±43 (4)

fatty acid synthetase and 'malic' enzyme all had higher activities in the groups fed on the fat-free or high-fat diets than in the group fed on the chow diet (Table 5). These findings confirmed the trend indicated by the metabolic experiments performed with slices.

The observation that α-glycerol phosphate dehydrogenase activity was significantly lower in mammary glands from lactating than from virgin mice was somewhat unexpected in view of the results obtained by Baldwin & Milligan (1966). These workers showed that, in rats, the activity of this mammary-gland enzyme increased considerably during the early stages of lactation. In the virgin mammary gland adipose cells are the predominant cell type and, as they lack glycerol kinase (ATP–glycerol phosphotransferase, EC 2.7.1.30) activity (Wieland & Suyter, 1957), they must synthesize all their α-glycerol phosphate from dihydroxyacetone phosphate. In the lactating mammary gland, however, the mammary parenchymal cells are the predominant cell type and they do possess glycerol kinase activity. McBride & Korn (1964) have indeed shown that the glycerol kinase reaction can supply a significant proportion of the α-glycerol phosphate requirement of the lactating guinea-pig mammary gland. The difference in response of α-glycerol phosphate dehydrogenase to the physiological state of the gland may therefore be due to the different species used by Baldwin & Milligan (1966) and ourselves.

Lipid composition of milk and mammary glands

The effect of the various diets on the chain length of fatty acids synthesized by the lactating mammary gland and on the composition of milk fatty acids was also examined (Table 6). The major difference in the milk from the three groups was in their content of linoleate. This was more or less as expected as the three diets contained different amounts of linoleate (0, 3·1 and 9·2g./100g. of diet in the fat-free, chow and high-fat diets respectively). The total amount of fat in the milk samples from each of the three groups reflected to some extent the fat content of the diet, being lowest in the group fed on the fat-free diet and highest in the group fed on the high-fat diet. The amount of shorter-chain

Table 6. *Effect of diet on milk fat composition and type of fatty acids synthesized by lactating mammary gland*

Milk fatty acid compositions are expressed as moles/100 of total lipid and are given as means + S.E.M. of between five and seven samples. Fatty acids synthesized by the lactating-mammary-gland slices are expressed as the percentage of total radioactivity incorporated from [6-^{14}C]glucose. Results are given as the means of two determinations.

	Chow diet		Fat-free diet		High-fat diet	
Fatty acid	Milk fat composition	Fatty acids synthesized	Milk fat composition	Fatty acids synthesized	Milk fat composition	Fatty acids synthesized
$<C_8$	0	0	0	0	0	0
$C_{8:0}$	0·8±0·1	0·8	0·8±0·0	1·6	0·7±0·1	0
$C_{10:0}$	10·2±0·5	12·3	9·2±0·8	13·2	8·0±0·5	13·9
$C_{12:0}$	13·1±0·5	18·2	14·3±0·9	17·1	9·8±0·4	18·4
$C_{14:0}$	12·5±0·3	17·9	18·4±0·9	17·8	10·5±0·5	21·0
$C_{14:1}$	0·7±0·0		0·9±0·1		0·4±0·1	
$C_{16:0}$	22·0±0·7	22·4	27·7±1·3	23·6	18·0±0·9	28·9
$C_{16:1}$	2·7±0·2		6·4±0·3		1·6±0·2	
$C_{18:0}$	2·9±0·1		1·7±0·1		1·8±0·1	
$C_{18:1}$	20·1±0·1	13·7	18·2±1·4	11·7	16·0±0·3	7·7
$C_{18:2}$	14·5±0·5		1·7±0·7		32·4±0·8	
$C_{18:3}$	0·8±0·1		0·5±0·1		1·1±0·2	
$>C_{18}$	—	14·6	—	8·8	—	4·7
g. of fat/100g. of milk	16·7±1·1		13·5±0·8		19·0±0·6	

Table 7. *Effect of diet on fatty acid composition of livers and mammary glands from lactating mice*

Fatty acid compositions are given as moles/100 moles of total fatty acid ± S.E.M. A number of minor components (< 1%) were measured, but for the sake of simplicity are not reported.

	Liver			Mammary gland		
Fatty acid	Chow diet	Fat-free diet	High-fat diet	Chow diet	Fat-free diet	High-fat diet
$C_{10:0}$	0	0	0	4·5±1·4	4·6±0·6	1·4±0·3
$C_{12:0}$	0·1±0·0	0·1±0·0	0·1±0·0	5·9±1·6	9·8±1·0	4·6±0·6
$C_{14:0}$	0·6±0·1	0·6±0·1	0·4±0·1	7·1±1·3	12·9±1·5	7·2±0·8
$C_{16:0}$	21·7±0·5	23·9±0·8	13·7±3·2	23·6±1·6	27·3±2·6	22·4±0·8
$C_{16:1}$	2·4±0·3	4·0±0·7	1·8±0·2	3·6±0·4	5·6±0·6	2·0±0·1
$C_{18:0}$	12·0±1·3	11·9±0·5	8·6±3·7	6·7±0·3	4·8±1·0	3·9±0·3
$C_{18:1}$	24·9±1·8	28·8±1·5	19·0±1·4	28·4±3·1	24·3±1·4	22·6±0·7
$C_{18:2}$	14·4±1·1	6·5±1·1	24·2±2·4	13·4±0·8	4·2±1·5	33·4±1·5
$C_{18:3}$	0·4±0·1	0·2±0·1	0·3±0·1	0·4±0·0	0·1±0·0	0·4±0·2
$C_{20:4}$	16·8±2·1	14·3±1·4	26·8±2·8	1·4±0·5	4·1±0·4	1·5±0·1
Lipid (% of total wet wt.) ...	3·2±0·2	3·4±0·5	3·1±0·3	11·0±2·8	6·5±0·9	9·8±0·8
No. of determinations ...	3	3	3	3	7	4

fatty acids in the milk samples was very similar in each of the three groups (Table 6), consistent with the view that synthesis of these acids by the mammary gland was unaffected by the dietary manipulations. This was further indicated by results showing that the chain lengths of the fatty acids synthesized by lactating mammary-gland slices were quite similar in each of the three dietary groups (Table 6).

The fatty acid composition of liver and mammary gland from lactating mice showed similar trends in animals from the various dietary groups (Table 7). Mice transferred from the chow to the fat-free diet showed dramatic decreases in the linoleate content of each of the tissues, whereas those transferred to the high-fat diet showed increases in the linoleate content. The total lipid content of the livers remained unaltered by the dietary manipulations whereas the lipid content of mammary tissue was significantly lower in the group fed on the fat-free diet.

In virgin animals the total lipid content of the livers and mammary glands was unaffected by diet (Table 8). However, whereas the liver content was

Table 8. *Effect of diet on fatty acid composition of livers and mammary glands from virgin mice*

Fatty acid compositions are presented as moles/100 moles of total fatty acid ± S.E.M. A number of minor components (< 1%) were measured, but for the sake of simplicity are not reported. In mammary glands no fatty acids of chain length < C_{14} were detected.

	Liver			Mammary gland		
Fatty acid	Chow diet	Fat-free diet	High-fat diet	Chow diet	Fat-free diet	High-fat diet
$C_{14:0}$	0·4 ± 0·2	0·5 ± 0·1	0·3 ± 0·1	1·8 ± 0·1	1·8 ± 0·1	1·5 ± 0·2
$C_{16:0}$	15·1 ± 3·8	17·5 ± 2·0	14·4 ± 3·1	20·8 ± 0·7	20·9 ± 0·8	21·2 ± 0·2
$C_{16:1}$	2·7 ± 0·4	3·2 ± 0·6	2·3 ± 0·2	5·5 ± 0·3	6·9 ± 0·5	5·5 ± 1·1
$C_{18:0}$	9·3 ± 3·1	7·3 ± 2·3	6·3 ± 1·9	4·6 ± 0·3	3·8 ± 0·2	4·2 ± 0·3
$C_{18:1}$	24·2 ± 1·7	37·8 ± 3·9	22·0 ± 2·2	38·1 ± 1·9	39·1 ± 0·6	34·1 ± 1·6
$C_{18:2}$	18·4 ± 2·2	10·6 ± 1·6	27·9 ± 2·1	18·5 ± 0·8	15·8 ± 0·2	24·4 ± 0·9
$C_{18:3}$	0·6 ± 0·1	0·2 ± 0·1	0·4 ± 0·1	1·4 ± 0·0	1·1 ± 0·0	1·1 ± 0·2
$C_{20:4}$	21·2 ± 4·4	15·6 ± 1·8	19·2 ± 1·7	1·2 ± 0·3	1·5 ± 0·6	1·2 ± 0·1
Lipid (% of total wet wt.) ...	3·8 ± 0·2	3·3 ± 0·6	3·8 ± 0·4	48·0 ± 10·3	46·9 ± 2·8	49·4 ± 3·9
No. of determinations ...	3	3	3	2	3	3

identical in virgin and lactating animals, the lipid content of virgin mammary glands was much higher than that of lactating glands. This was to be expected, as the virgin gland is composed mainly of adipose cells. The change in liver linoleate content caused by transferring virgin mice to fat-free and high-fat diets was much the same as that found with lactating mice. The linoleate content of mammary gland, however, was much less affected by the dietary changes in virgin mice than it was in lactating mice.

DISCUSSION

It is well established that diets rich in fat lead to decreased lipogenesis in the liver of non-lactating animals. The results of this study show that the rate of hepatic lipogenesis is also decreased in lactating mice fed on a high-fat diet and that there are corresponding adaptive changes in the activity of certain hepatic enzymes. The fact that the rate of hepatic fatty acid synthesis in mice fed on the high-fat diet did not fall below the rate found in mice fed on a chow diet may well be accounted for by the relatively high fat content of chow.

The increase in the rate of lipogenesis in mammary gland that occurred when virgin animals were transferred from a chow diet to either a fat-free or a high-fat diet was surprising in view of the fact that virgin mammary gland is composed almost entirely of adipose cells. A similar observation was made by Jansen, Hutchison & Zanetti (1966), who studied lipogenesis *in vivo* in young adult male mice. Jansen *et al.* (1966) found that incorporation of orally administered [U-^{14}C]glucose into epididymal fat-pads was increased when mice were transferred from a chow diet to a corn-oil-supplemented (1–20%) diet, but they offered no explanation for this finding. The effect may possibly be related in some way to the nature of the carbohydrate material in chow and corn-oil-supplemented diets. The carbohydrate of the chow diet consists mainly of polysaccharides whereas glucose was the sole dietary carbohydrate in the corn-oil-supplemented diets used by Jansen *et al.* (1966) and ourselves. In our study such a stimulation was not observed in liver, as there was no significant difference in hepatic lipogenesis between animals fed on the chow and the high-fat diets. However, Hill *et al.* (1958) showed that dietary fat decreased hepatic lipogenesis even when the diet contained 50% of glucose, i.e. under conditions that allowed for priming of hepatic lipogenesis by dietary carbohydrate. These workers concluded that the lipogenic capacity of the liver was more sensitive to dietary fat than to dietary carbohydrate. In our study we have shown that adipose tissue of C_3H mice is relatively insensitive to dietary fat when glucose is the sole dietary source of carbohydrate. Thus in virgin mice transferred from the chow to the high-fat diet the lipogenic capacity of mammary-gland adipose tissue is increased, possibly owing to the change in the type of dietary carbohydrate, whereas in liver the effect is not observed because of the extreme sensitivity of this tissue to the inhibitive effect of dietary fat.

The observation that a high-fat diet does not decrease the rate of fatty acid synthesis in mammary-gland slices from virgin animals to below the rate found in animals fed on a fat-free diet prompted further investigation to determine whether, in virgin C_3H mice, the adipose tissue of mammary gland responded differently from adipose tissue from another part of the body. Animals fed on chow diet were transferred to either the high-fat or fat-free diets for 3 days and a study was made of lipogenesis in the uterine fat-pad. Fatty acid synthesis from

[1-^{14}C]- and [6-^{14}C]-glucose by slices of uterine adipose tissue was markedly higher in animals fed on either the high-fat or the fat-free diet than in those fed on the chow diet. Further, in animals fed on the high-fat diet the rate of fatty acid synthesis was not depressed below the rate found in animals fed on the fat-free diet. The activities of fatty acid synthetase, citrate-cleavage enzyme, 'malic' enzyme and acetyl-CoA carboxylase were approximately the same in animals fed on the fat-free and the high-fat diets, but considerably lower in animals fed on the chow diet (S. Smith & S. Abraham, unpublished work). Thus adipose tissue from both the uterine fat-pad and the virgin mammary gland appears to respond in the same way to the various dietary changes. These results emphasize the point that, in the mouse, adipose tissue does not necessarily adapt to dietary changes in the same way as does the liver.

Fatty acid synthesis in the lactating mammary gland was identical in animals fed on the chow, the high-fat and the fat-free diets, in contrast with the virgin gland, which showed decreased rates of synthesis in animals fed on the chow diet. The different response of the lactating and virgin mammary gland to dietary changes could be due either to the changed hormonal environment or to the change in the predominant cell type. Hepatic lipogenesis is to some extent influenced by the transition from the virgin to the lactating state; incorporation of acetate into fat is twice as high in liver slices from lactating animals as in those from virgin animals fed on chow diets, and the activities of several enzymes are considerably increased. Nevertheless, the liver retains its ability to adapt to dietary changes in the lactating condition. We therefore tend to favour the hypothesis that the lack of response of the lactating mammary gland to dietary changes is specifically a property of the mammary-gland parenchymal cell rather than of the different hormonal status of the animal during the lactational period.

The apparent adaptive nature of citrate-cleavage enzyme in the lactating mammary gland merits comment. Although changes in rates of fatty acid synthesis are closely associated with changes in the activity of cirtate-cleavage enzyme in a tissue (Kornacker & Lowenstein, 1964, 1965; Kornacker & Ball, 1965), the possibility that the activity of this enzyme might in fact directly control the rate of fatty acid synthesis on a short-term basis has been discounted by Foster & Srere (1968). These workers have shown that in starved and re-fed rats hepatic lipogenesis increased several hours before any change in the activity of citrate-cleavage enzyme could be observed. Results given in the present paper illustrate yet another instance of a non-correlation between these two events, but this time on a long-term basis. Here we see that an adaptive change in citrate-cleavage enzyme activity is not accompanied by a change in the rate of fatty acid synthesis over a period of 3 days.

The activity of acetyl-CoA carboxylase was always the lowest of the enzyme activities measured in liver or mammary gland. We calculated that under all of the dietary conditions the activity of this enzyme in either liver or mammary gland was always sufficient to account for the observed rate of fatty acid synthesis in tissue slices. Nevertheless, changes in the activity of acetyl-CoA carboxylase were not always exactly parallel by changes in the rate of fatty acid synthesis. Measurements of enzyme activities *in vitro* suggest that the rate of acetyl-CoA carboxylation might be the rate-limiting step in fatty acid synthesis. However, from these results alone it cannot necessarily be implied to be rate-controlling *in vivo*.

Allman & Gibson (1965), using young male mice, showed that when the animals were transferred to a fat-free diet the linoleate content of the liver decreased much more rapidly than that of epididymal adipose tissue. Our experiments show that the linoleate content of both liver and mammary gland was drastically decreased when lactating animals were transferred to a fat-free diet for 3 days. When virgin mice were used, changes in liver linoleate were much less marked and the linoleate content of mammary gland was only slightly affected. The relatively rapid loss of linoleate from tissues of lactating compared with virgin animals transferred to a fat-free diet may be a consequence of the additional demand for this fatty acid in milk production. It is noteworthy that in virgin animals the linoleate content of mammary gland appears to be much less affected than that of the liver by the dietary fat content. Since the virgin gland is composed mainly of adipose cells our results on the changes in linoleate content in virgins are consistent with the observations of Allman & Gibson (1965) cited above.

Allman & Gibson (1965) demonstrated a strong correlation between dietary linoleate, tissue linoleate content and rate of fatty acid synthesis in liver and adipose tissue. We have shown that no such correlation is evident in lactating mammary gland. The linoleate content of lactating mammary gland responds to changes in dietary fat in a similar way to that of the liver, but whereas in liver the changes were accompanied by alteration in the fat-synthesizing capacity, in the mammary gland the fat-synthesizing capacity remained unchanged.

Adipose tissue from the mammary gland and uterine fat-pad of virgin C_3H mice differs from the epididymal adipose tissue of the C57 Bl/6J mice studied by Allman & Gibson (1965) in that it does not show decreased lipogenic capacity in animals fed

on a corn-oil-supplemented diet compared with animals fed on a fat-free diet (Hausberger & Milstein, (1955). Favarger (1965), Leveille (1967) and Jansen, Zanetti & Hutchison (1967) have estimated that in rats and mice liver accounts for less than 10% of the total fatty acids synthesized by the body. Farvager (1965) and Leveille (1967) have calculated that 50–90% of the fatty acids synthesized are produced by adipose tissue. From the present results we have estimated that in both virgin and lactating C_3H mice fed on a chow diet the total fatty acid-synthesizing capacity of the mammary gland is greater than that of the liver. As fatty acid synthesis in uterine adipose tissue of C_3H mice also appears to be insensitive to the dietary fat content (S. Smith & S. Abraham, unpublished work), the C_3H mouse presents a situation where only a very small proportion of its lipogenic capacity is adaptive to the fat content of the diet.

The inflexible nature of the lactating-mammary-gland lipogenic system towards dietary adaptation that we have demonstrated here may ensure continued production of milk containing the fatty acids C_{10}–C_{14}, characteristic of that species. The long-chain fatty acid components of milk fat may be maintained, at least to some extent, as a result of the adaptive capacity of the liver. Thus lactating animals feeding on a fat-free diet would rely on increased hepatic lipogenesis for a continued supply of the long-chain fatty acid component of milk fat. Conversely, animals fed on a high-fat diet would be able to take a correspondingly larger proportion of their long-chain requirement from dietary fatty acids, thereby decreasing the demand for hepatic fat synthesis.

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