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The influence of diet and diabetes on stearoyl Coenzyme A desaturase (*EC* 1.14.99.5) activity and fatty acid composition in rat tissues

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1. Rats were given low-fat diets for 3 d in which the carbohydrate source was starch. The livers of animals given the fructose or sucrose had increased hepatic activities of the fatty acid synthetase and stearoyl CoA desaturase (*EC* 1.14.99.5) enzyme complexes: in those given fructose there was a lower activity of the enzymes in adipose tissue.

2. Similar results were obtained in rats given fructose diets for 30 d, but in animals which had previously been made diabetic with streptozotocin, the activities were lower. The dietary treatment made little difference to the fatty acid profiles of the tissue lipids. The diabetic condition on the other hand produced considerable changes in fatty acid profile.

3. With diets containing approximately 200 g fat/kg in the form of butter or of polyunsaturated margarine, the tissue lipids from rats given sucrose had less linoleic acid than those from rats given starch. In addition, there was the expected difference between the rats given butter or margarine. The results are discussed in relation to the current literature.

In many tissues the Δ9 desaturase enzyme complex converts saturated fatty acyl coenzyme A esters to the monounsaturated form. In rats this enzyme is increased by saturated fat in the diet and decreased by fasting (Inkpen *et al.* 1969), so that monounsaturated fatty acids occur in high concentration in most lipid fractions. Whereas there is considerable evidence that dietary fructose or sucrose increased hepatic fatty acid synthesis and associated enzymes (Bruckdorfer *et al.* 1972; Cohen, *et al.* 1972; Romsos & Leveille, 1974), the effects of different dietary carbohydrates on desaturation have been much less studied. Early work indicated that feeding sucrose to rabbits or human volunteers resulted in changes in the fatty acid profile of tissue lipids, primarily a decrease in the linoleic acid content and a concomitant increase in the monounsaturated fatty acids (Macdonald, 1962; Macdonald & Braithwaite, 1964). Similar changes were shown in the biliary phosphatidylcholines of human subjects given sucrose, although in that study the dietary controls were not adequate (Alling *et al.* 1973). In more recent experiments with rats, dietary fructose produced greater activity in hepatic stearoyl CoA desaturase (*EC* 1.14.99.5) both in vivo and in vitro than did dietary glucose (Mercuri *et al.* 1974). These experiments were short term and the rats were given the glucose and fructose diets for different periods of time, giving rise to the possibility that the differences were due to 'overshoot effects' as a result of diet change. No comparison of the more usual dietary carbohydrates starch and sucrose was made in that study.

It has also been shown that fructose and sucrose have opposite effect on adipose tissue, in that they decrease lipogenesis and lipogenic enzyme activities (Bruckdorfer *et al.*, 1972; Romsos & Leveille, 1974). For this reason we examined the effects of sucrose and fructose

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on desaturation in adipose tissue as well as in the liver. De Tomas *et al.* (1973) found that desaturase activity was reduced in rats made diabetic with streptozotocin, suggesting that the enzyme was controlled by insulin as was suggested in the earlier work of Gellhorn & Benjamin (1964), who used alloxan diabetic rats. We therefore investigated the effects of fructose in rats treated with strepozotocin.

A simple assay system has been devised for stearoyl CoA desaturase (Johnson & Gurr, 1971) in which the crude microsomal preparation is incubated with [9,10^{3}H$_2$(*n*)]stearic acid dispersions, which results in the release of tritium-labelled water in proportion to the enzyme activity. One of the difficulties with this procedure is the preparation of consistent dispersions of stearic acid. In this paper we describe a modification of this method, in which stearic acid was co-dispersed with phosphatidylcholine by ultrasonication.

The fatty acid composition of tissue lipids in long-term experiments with fructose and sucrose, sometimes with fat-rich diets, were determined.

MATERIALS AND METHODS

Animals and diets

Male Sprague-Dawley rats (Charles River (UK) Ltd, Margate, Kent) weighing 100–110 g were given standard laboratory diet 41B (Dixon Ltd, Ware, Herts.) for 1 week before feeding experimental diets *ad lib.* for 3 or 30 d or 6 months with free access to water. Some rats were made diabetic by injection of streptozotocin (Upjohn Ltd, Kalamazoo, USA) into the tail vein in a dose of 65 mg/kg body-weight. Glycosuria was detected with 'Clinistix' (Ames Co., Slough, Bucks.) and the animals given the experimental diets after 1 week. The rats were randomized into groups which were given diets differing by the type of carbohydrate, and in one experiment also by the nature of the fat.

The low-fat diet contained (g/kg): 700 fructose, glucose, sucrose or maize starch, 190 casein (Casumen; Unigate Food Ltd, Trowbridge, Wilts.), 20 maize oil, 30 cellulose, 20 vitamin mix and 40 mineral mix. The vitamin mix contained cellulose, choline bitartrate and vitamin concentrate in the proportion of 1760:180:60·2 by weight. The vitamin concentrate contained (g): ascorbic acid 15, nicotinic acid 12, vitamin B_{12} mix 10 (1 mg cyanocobalamin/g), calcium pantothenate 8, thiamine hydrochloride 2, riboflavin 2, pyridoxine 2, folic acid 1, biotin 0·29, menadione 0·2, Rovimix E 25 60, Rovimix AD_3 500/100 2·4, Rovimix A-500 2·8 (Roche Products Ltd, Welwyn, Herts). The mineral mix contained: $CaHPO_4.2H_2O$, $CaCO_3$, KCl, Na_2HPO_4, $MgSO_4.7H_2O$, trace minerals in the proportions 1644:820:740:498·5:46·5, by weight. The trace minerals were $MnSO_4.4H_2O$, ferric citrate, $CuSO_4.5H_2O$, $ZnCO_3$ and KIO_3 in the proportions 59·4:43·5:5.9:7.5:0·25, by weight.

The fat-rich diets contained (g/kg) 550 sucrose or starch, 200 butter or polyunsaturated margarine, 160 casein and the same ingredients as in the low-fat diet but excluding maize oil. Animals on these diets were fed for a period of 6 months.

At the end of the experiments the animals were killed by carbon dioxide anaesthesia and in some instances exsanguinated from the heart. Livers and epididymal adipose tissues were also excised, washed in cold isotonic saline (9 g sodium chloride/l), blotted and weighed. Portions of the tissues were frozen for the later determination of lipid fatty acid composition.

Other tissue samples were weighed fresh and homogenized in 0·25 M-sucrose and centrifuged at 10 000 *g* for 10 min at 4 ° in an MSE 18 centrifuge (MSE Scientific Instruments, Crawley, Sussex). The supernatant fraction was then centrifuged for 1 h at 4 ° in an MSE 50 Ultracentrifuge at 104 000 *g* and the microsomal pellet and supernatant fraction retained. The microsomal pellet was either frozen at −21 ° or used directly by resuspension in 0·25 M-sucrose (pH 7·4) and diluted to 2·5 mg protein/ml (Lowry *et al.* 1951).

Fatty acid synthetase

The activity was assayed on the 104000 *g* supernatant fraction as described in a previous paper (Bruckdorfer *et al.* 1972). Malonyl CoA, acetyl CoA, NADPH and bovine serum albumin (fatty acid free) were purchased from Sigma Chemical Co., London.

Stearoyl CoA desaturase

The method adopted was based on that described by Johnson & Gurr (1971) in which the elimination of tritium into water from [9,10$^3H_2$(*n*)]stearic acid (Radiochemical Centre, Amersham, Bucks.) was used to measure activity in crude microsomal preparations. The main difficulty with this method was an inconsistency in the dispersion of stearic acid in an aqueous environment. This can be improved with sodium hydroxide or detergents, but consistent and reproducible values were obtained by dispersion of the stearic acid in egg phosphatidylcholine liposomes.

Preparation of liposomes. 0·6 μmol(100 μc) [9,10$^3H_2$(*n*)]stearic acid and 6·0 μmol egg phosphatidylcholine (Lipid Products, Redhill, Surrey) were dried under nitrogen in a vial which was sealed after addition of 2 ml 0·02 M-hepes buffer, pH 7·4. The vial was sonicated for 4 min in a sonication bath (Rapidis Ultrasonic Disintegrator 20 KHz, 150 watt; Ultrasonics Ltd, Shipley). Short periods of sonication were found to be adequate for dispersion of the stearic acid and 'background' radioactivity due to tritium exchange increased with longer sonication. This gave enough substrate for approximately twenty assays. Dispersions were prepared fresh each day to reduce tritium exchange with the aqueous medium.

Incubation with microsomes. Each assay mixture was prepared as follows: 0·1 ml stearic acid dispersion (5 μC) was pipetted into a small stoppered tube and mixed with other solutions to give a volume of 0·25 ml, containing concentrations of 3·3 mM-Mg^{2+}, 0·134 mM-coenzyme A, 16 mM-ATP and 0·934 mM-NADH. To this ice-cooled mixture 0·2 ml microsomal suspension (usually 0·5 mg protein) was added, mixed for 5 s and then incubated in a water bath at 37 ° for 5 min.

After 10 min the reaction was halted with 0·05 ml 40 mM-potassium cyanide containing 28·8 g bovine serum albumin/l, which both inhibit the enzyme through the cyanide-sensitive component of the electron transport chain and bind the stearic acid substrate. After 30 s, 1 ml trichloroacetic acid (100 g/l) was added, precipitating protein and bound-lipid and leaving tritiated water due to desaturation in the aqueous medium. After 10 min in ice the tubes were centrifuged for 10 min at 3000 *g*. Supernantant fraction (1 ml) was transferred to a scintillation vial and dispersed in 10 ml of a mixture (2:1, by vol) of scintillation fluid (6 g 2(4′-*t*-butylphenyl)-5-(4″-biphenyl)-1,3,4,-oxadiazole and 0·1 g 4 methyl 1,4 di 2-(4 methyl-5 phenyloxazole)-benzene in 1 l toluene: Koch Light Laboratories Ltd., Colnbrook, Bucks.) – Triton X-100 (Koch Light Ltd), and radioactivity counted on a Packard Tricarb scintillation spectrometer (Model 3385). Each assay was performed in triplicate. Addition of internal standards to a number of assays showed a very consistent efficiency of counting from sample to sample (35·3%).

Control assays were performed in duplicate for each sample. This was achieved by addition of the cyanide before the incubation, which inhibited enzyme activity but not the tritium exchange between stearic acid and water. In most instances the 'background' counts were less than 25% of the total counts. After subtraction of the 'control' counts, the activity was expressed as counts/min per mg microsomal protein per min. The absolute activity of the enzyme could not be readily calculated because of the mixed substrates used and existence of isotope effects (Johnson & Gurr, 1971). Nevertheless these authors have shown that oleate formation is proportional to tritiated water release.

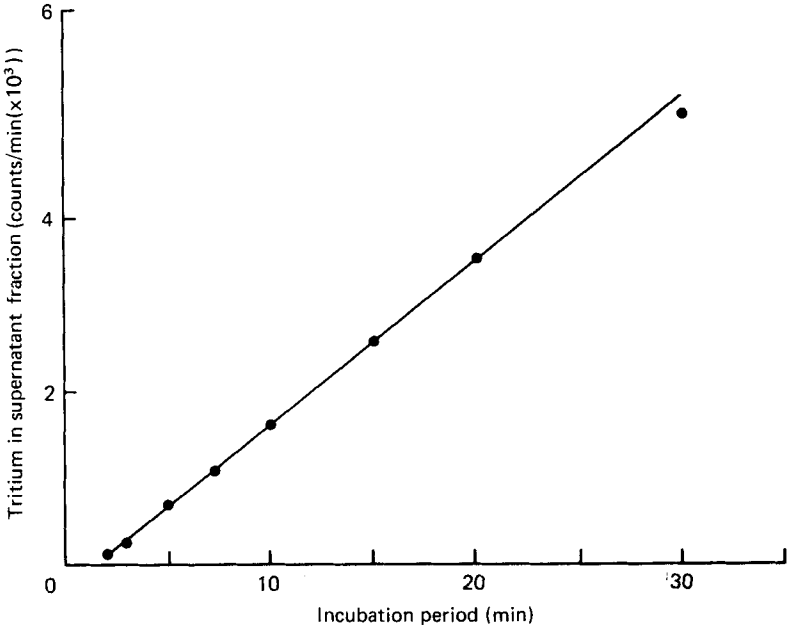

Fig. 1. Reliability studies on stearoyl CoA desaturase activity. Liposomes containing 0·03 μmol (5 μc) of [9,10^{3}H$_2$(n)]stearic acid and 0·3 μmol egg phosphatidylcholine in hepes buffer pH 7·4 were incubated for 5 – 30 min with rat liver microsomes (0·5 mg protein) in a medium containing Mg^{2+}, coenzyme A, ATP and NADH to permit formation of stearoyl CoA and its desaturation. The reaction was stopped by addition of potassium cyanide and bovine serum albumin and the ^{3}H eliminated during desaturation as ^3H_2O was counted in the supernatant fraction obtained after addition of trichloroacetic acid and centrifugation (for details, see p. 241). A blank value was obtained from incubations which were identical except that KCN was added at the beginning. The points are an average of triplicate measurements.

Insulin

Plasma insulin concentrations were determined by the radioimmunoassay procedure of Hales & Randle (1963). Materials were obtained from the Radiochemical Centre, Amersham, Bucks. Values are expressed in equivalents of porcine insulin.

Lipid analysis

Lipids in the plasma, adipose tissue and liver were extracted by the method of Folch *et al.* (1957), and the major fractions containing fatty acid separated by thin-layer chromatography (Skipski *et al.* 1968). Methyl esters of the fatty acids contained in the lipid fractions were prepared with methanolic hydrogen chloride or sodium methoxide as described by Christie (1973). The fatty acid composition was determined using a Pye 104 gas chromatograph with dual flame ionization detection (Pye-Unicam Ltd, Cambridge), operating at 190 ° with an argon flow of 50 ml/min through 1·5 m glass columns containing 100 g polyethyleneglycoladipate/kg 100 – 120 Mesh Diatomite CAW support (Pye Unicam Ltd). Peak areas were determined directly by a computing integrator DP 88 (Pye Unicam Ltd).

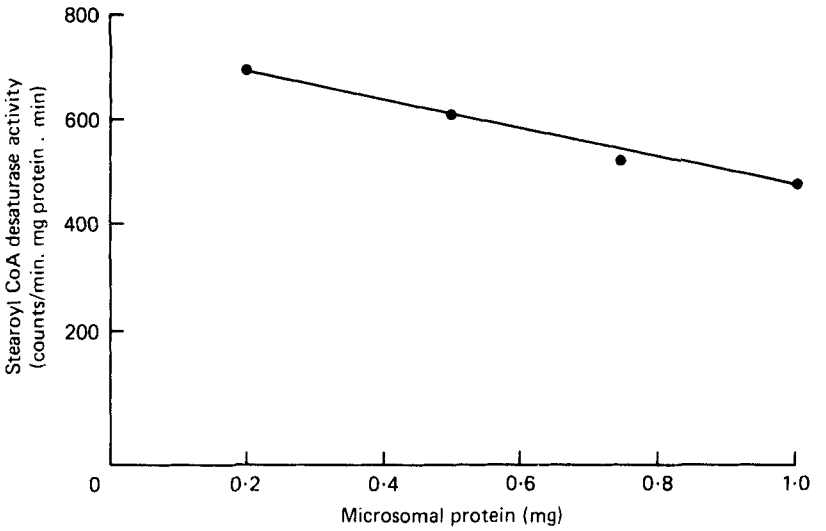

Fig. 2. Stearoyl coenzyme A desaturase activity (counts/min per mg protein per min) at a range of microsomal protein concentrations (for details of procedures, see p. 241). The activity was expressed as ^3H label released per mg microsomal protein. The points are an average of triplicate measurements.

RESULTS

Stearoyl CoA desaturase assay

Experiments were performed to demonstrate the reliability of this assay procedure. With a fixed quantity of microsomes, 0·5 mg protein/ml, a linear time-course for activity was obtained for at least 30 min (Fig. 1) and a 10 min interval was used for further assays. A large excess of substrate was used and the activity appeared to vary only slightly in the range 0·2 – 1·0 mg microsomal protein (Fig. 2). Nevertheless in all assays the microsomal protein was diluted to give a final concentration of 0·5 mg protein/ml. Reproducible results were obtained in triplicated assays on the same microsomes under identical conditions, giving a value with the range $\pm 4\%$ of the mean.

Short-term experiments

Rats were fed on a low-fat diet containing fructose or glucose for 3 d as described by Mercuri *et al.* (1974). As observed in earlier work (Bruckdorfer *et al.* 1972), the liver weight was greater in the animals fed on fructose compared to those fed on glucose (Table 1(*a*)). In the liver the activities of both fatty acid synthetase and stearoyl CoA desaturase were significantly increased by feeding fructose. In adipose tissue, fatty acid synthetase was lower in the fructose group as previously observed (Bruckdorfer *et al.* 1972). Stearoyl CoA desaturase activity was also lower in rats fed on fructose, but to a smaller extent.

With diets containing sucrose and starch, differences were again observed. In the 3 d of this experiment, sucrose did not produce the greater liver weight seen in more prolonged experiments (Bruckdorfer *et al.* 1972). Hepatic fatty acid synthetase activities showed a greater difference between the starch and sucrose groups than did the activities of stearoyl CoA desaturase; for both enzymes the differences were statistically significant (Table 1(*b*)). In the adipose tissue only fatty acid synthetase activity was significantly lower in the sucrose group.

Table 1. *The activities of stearoyl CoA desaturase* (EC 1.14.99.5) (*counts/min per mg protein per min*) *and fatty acid synthetase* (*μmol NADPH/min per g tissue*) *in liver and adipose tissue of rats given diets containing* (*a*) *fructose or glucose or* (*b*) *sucrose or starch for* 3 *d*†

(Mean values and standard deviations for eight rats/group. No differences in body-weight were noted between the groups which were compared)

	(*a*)				(*b*)			
	Fructose		Glucose		Sucrose		Starch	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Liver wt (g)	8·01	0·53	6·86	0·48**	8·60	0·90	7·40	1·10
Liver:								
Stearoyl CoA desaturase	595	40	431	73**	857	65	770	26**
Fatty acid synthetase	3·90	0·28	2·83	0·37***	6·35	0·64	2·48	0·62***
Adipose tissue:								
Stearoyl CoA desaturase	228	13	273	11***	196	35	217	36
Fatty acid synthetase	0·109	0·090	0·379	0·170**	0·114	0·022	0·191	0·037

The difference between groups was significant (Students' *t* test).
* $P<0·05$, ** $P<0·01$, *** $P<0·001$.
† For details of diets, see p. 240.

Fructose and diabetes

The comparison of the effects of low-fat fructose and glucose diets was also made after a period of 30 d. These diets were given to animals made diabetic with streptozotocin. Fructose again produced heavier livers in both normal and diabetic rats (Table 2). As in the short-term experiment, fructose increased the activities of hepatic fatty acid synthetase and stearoyl CoA desaturase in normal rats and also in diabetic rats. Diabetes decreased these enzyme activities in rats fed on both diets (Table 2). The adipose tissue mass of diabetic rats was too small for enzyme activity to be measured. A greater difference between fructose- and glucose-fed rats was observed for fatty acid synthetase than in the 3 d experiment, but the decrease in desaturase activity in fructose-fed rats was less, although still significant.

In the same experiment very low concentrations of plasma insulin levels were detected in the diabetic rats, but no significant differences were caused by the different dietary treatments. When the values for all groups were combined a positive correlation, r 0·73 ($P < 0·001$), was found between plasma insulin and liver fatty acid synthetase activities, but not between insulin and any other measurements. A correlation also exists between hepatic fatty acid synthetase and stearoyl CoA desaturase activity both in liver, r 0·53 ($P < 0·01$), and also in adipose tissue r 0·53 ($P < 0·02$). Similar correlations were observed in the 3 d experiment between the two enzymes in liver, r 0·64 ($P < 0·01$), and in adipose tissue r 0·59 ($P < 0·01$).

Fatty acid profiles

Analyses were made of the tissue fatty acid profiles of rats fed on the low-fat diets for 30 d and of those fed fat-rich diets for 6 months. The effects of the carbohydrate component of the diets were greater in the long-term experiment.

Only slight differences occurred in the fatty acid compositions of the phospholipid and triglyceride fractions of plasma and liver lipids obtained from rats fed on fructose and glucose diets (Tables 3 and 4). This was also true of the cholesteryl ester fatty acids and the total lipid profiles from adipose tissue (not shown). The values for the ratios, 16:0:16:1 fatty acids and 18:0:18:1 fatty acids were also not significantly different. As may be expected in animals where the metabolism of lipids has been profoundly disturbed, fatty acid composition of the plasma and liver lipids of the lean diabetic rats were very different, irrespective of the dietary carbohydrate, with a much larger content of polyunsaturated fatty acids, especially linoleic acid. In all instances, with the exception of plasma phospholipids, there was an increase in the value for 18:0:18:1 fatty acids indicating impaired desaturation in diabetes (Tables 3 and 4). The value for 16:0:16:1 fatty acids was not measurable in all fractions because of the very low content of 16:1, especially in diabetic rats. In liver triglycerides, no significant change in the value for 16:0:16:1 was observed, but in liver phospholipids and plasma triglycerides the virtual disappearance of the 16:1 (palmitoleic) fatty acid indicated a low level of desaturation of 16:0 in diabetes. In cholesteryl ester fractions a higher 16:0:16:1 value was found in diabetic rats (not shown).

In the long-term experiment with high levels of fat in the diet a clear effect of dietary carbohydrate and fat was shown. The linoleic acid contents of the diets differed considerably, since butter contained 25 g linoleic acid/kg and margarine 560 g linoleic acid/kg. Starch also has a small unextractable fraction of free linoleic acid so that the four diets contained slight unequal amounts of linoleic acid, even when the same dietary fat was used: (g/kg diet) sucrose–butter 5·0, sucrose–margarine 111·8, starch–butter 6·5, and starch–margarine 113·3.

Despite their presence in butter only traces of short- and medium-chain fatty acids were found in the tissues of rats which had been fasted overnight and then killed. The most striking difference between the groups of rats was the expected large amounts of linoleic

Table 2. *The activities of stearoyl CoA desaturase* (EC 1.14.99.5) (*counts/min per mg protein per min*) *and fatty acid synthetase* (*μmol NADPH/min per g tissue*) *in liver and adipose tissue of normal and diabetic rats given diets containing fructose or glucose for* 30 *d*§

(Mean values and standard deviations for eight rats/group)

Dietary Carbohydrate	Normal				Diabetic			
	Fructose		Glucose		Fructose		Glucose	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Liver wt g/kg body wt	51·7	2·9	41·4	2·9	58·5	10·7	43·9	3·9***†
Liver:								
Stearoyl CoA desaturase	513	28	393	25	485	75	337	65***†
Fatty acid synthetase	5·73	0·78	3·13	0·62	2·16	0·58	0·97	0·34***†††‡‡
Adipose tissue:								
Stearoyl CoA desaturase	209	12	259	25***				
Fatty acid synthetase	0·093	0·02	0·48	0·06***				
Plasma insulin (μU/ml)	31	13	22	13	2·6	3·5	2·1	2·0†††

The difference between treatment was significant (analysis of variance or Student's *t* test).
Fructose *v.* glucose, ***$P<0.001$.
Diabetic *v.* normal, † $P<0.05$, ††† $P<0.001$.
Interaction, ‡‡ $P<0.01$.
§ For details of diets and streptozotocin treatment, see p. 240.

Table 3. *Composition of fatty acids (mmol/mol) in liver and plasma phospholipids of normal and diabetic rats fed fructose and glucose diets§ for 30 d*

(Mean values and standard deviations for eight rats/group)

		16:0		16:1		18:0		18:1		18:2ω^6		20:4ω^6		18:0:18:1	
Liver	Group	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
	Fructose	232	22	33	5	296	34	143	16	96	11	200	40	2·1	0·4
	Glucose	247	24	36	8	279	36	144	26	99	16	194	40	2·0	0·6
	Fructose, diabetic	202	48	16	7	279	33	119	10	191	38	193	48	2·4	0·4
	Glucose, diabetic	204	34	trace		284	24	94	11	222	46	188	35	3·1	0·6
Analysis of variance		††		††	‡			†††	‡					†††	‡
Plasma	Fructose	303	46	trace		354	75	159	14	96	62	82	59	2·3	0·5
	Glucose	303	36	trace		257	52	147	36	163	52	119	41	1·9	0·8
	Fructose, diabetic	279	51	trace		269	30	132	24	241	52	78	41	2·2	0·8
	Glucose, diabetic	310	84	trace		254	54	109	22	246	90	80	65	2·4	0·7
Analysis of variance								*††		††					

Differences between values for fructose and glucose groups: * $P < 0.05$.
Differences between values for normal and diabetic groups: †† $P < 0.01$, ††† $P < 0.001$.
Interactions between dietary carbohydrate and diabetes were significant: ‡ $P < 0.01$.
§ For details of diets and streptozotocin treatment, see p. 240.

Table 4. *Composition of fatty acids (mmol/mol) in liver and plasma triglycerides of normal and diabetic rats given fructose and glucose diets§ for 30 d*

(Mean values and standard deviations for eight rats/group)

		14:0		16:0		16:1		18:0		18:1		18:2ω^6		16:0:16:1		18:0:18:1	
Liver	Group	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
	Fructose	16	3	440	30	71	9	38	13	418	38	16	8	6·4	1·3	0·09	0·04
	Glucose	13	2	493	40	73	18	40	13	361	41	21	9	7·4	2·6	0·11	0·06
	Fructose, diabetic	19	28	272	72	52	23	69	24	275	71	152	45	6·5	3·8	0·28	0·14
	Glucose, diabetic	17	17	315	55	58	27	62	30	272	54	135	71	8·0	7·3	0·23	0·11
Analysis of variance				*†††		†		††		†††		†††				†††	
Plasma	Fructose	25	12	344	62	71	20	45	20	429	58	88	27		—	0·11	0·07
	Glucose	30	11	355	45	88	25	46	14	432	60	48	16		—	0·11	0·04
	Fructose, diabetic	29	23	285	72	trace		89	39	344	72	236	79		—	0·28	0·14
	Glucose, diabetic	19	22	305	54	trace		104	65	321	77	232	98		—	0·37	0·26
Analysis of variance				†				†††		†††		†††				†††	

Differences between values for fructose and glucose groups: * $P < 0·05$.
Differences between values for normal and diabetic groups: † $P < 0·05$, †† $P < 0·01$, ††† $P < 0·001$.
§ For details of diets and streptozotocin treatment, see p. 240.

acid content and to a less extent of arachidonic acid in the tissue lipids from rats fed on margarine (Tables 5 and 6). There was a concomitant change in the proportion of most other fatty acids, particularly oleic and stearic acids, thus giving a higher value for 18:0:18:1 in margarine fed animals. Where sufficient 16:1 was detectable, the presence of margarine in the diet also gave a higher value for 16:0:16:1, although the proportion of 16:0 of the total is only slightly different in the tissue lipids of rats in the four dietary groups.

There were also differences in the fatty acid composition of rats fed on different carbohydrates. The main change was a lower percentage of linoleic acid in the tissues of sucrose-fed animals (Tables 5 and 6). This was statistically significant by analysis of variance in the plasma phospholipids and liver triglycerides and in the cholesteryl ester fractions of liver and plasma (not shown). In other fractions, liver phospholipid and plasma triglyceride and total adipose tissue lipid, there was a significantly lower linoleic acid content in rats fed on sucrose and butter compared to those fed on starch and butter when a Student's *t* test was used. In the presence of margarine the difference disappeared. Usually the difference between rats fed on sucrose or starch in linoleic acid content of tissue lipids was proportionally much greater than the small differences in the amount of this fatty acid in the diet. In most instances this decrease in linoleic acid was accompanied by an increase in oleic acid, but without a consistent change in the value of 18:0:18:1.

DISCUSSION

The assay system for stearoyl CoA desaturase proved to be reliable and reproducible, but there was some slight but linear variation in activity over a range of microsomal protein concentrations. In a recent study by Jeffcoat *et al.* (1976, 1977), in which a different assay system was used (^{14}C-stearoyl CoA substrate), it was found that the presence of bovine serum albumin or a purified soluble liver protein was necessary for a linear relationship between enzyme activity and protein concentration; this occurred in the absence of these agents only when the microsomes are partially purified.

Our own study confirmed previous reports that the activity of stearoyl CoA desaturase depends on the nature of the dietary carbohydrate not only in short 3 d experiments (Mercuri *et al.* 1974) but also after feeding for longer periods when 'overshoot' effects should have subsided. The changes in desaturase activity were in general paralleled by those of fatty acid synthetase, but the differences due to changes in dietary carbohydrate on the latter enzyme were usually proportionally greater. In adipose tissue a diet with fructose produced lower desaturase activity than did a diet with glucose, but dietary sucrose produced the same activity as did dietary starch.

Rats made diabetic with streptozotocin and given fructose or glucose had lower liver desaturase and synthetase activity than normal animals, but the difference was less than that reported by other workers (Mercuri *et al.* 1974). This could be accounted for by the longer duration of our experiment, although the insulin levels of the diabetic animals were very low. Fructose restored the activity in diabetic animals to a value above that of the normal glucose group. As shown in earlier work (Bruckdorfer *et al.* 1972), only by combining the results of all groups could a significant correlation be found between fatty acid synthetase activity and plasma insulin. This was also true for stearoyl CoA desaturase activity. This suggests that control by insulin may occur only in rather extreme conditions and that the fine regulation of these enzymes must be controlled by other factors, possibly as a group (Jeffcoat, 1977).

The close similarity of the fatty acid composition of tissue lipids in rats fed on fructose and glucose suggests that the desaturation of saturated fatty acids is linked to their increased synthesis, especially in the liver, as concluded by Wahle (1974*a*). The relationship between fatty acid synthesis and desaturation was also apparent in genetically-obese rats fed

Table 5. *Composition of fatty acids (mmol/mol) in liver and plasma phospholipids of rats given diets containing sucrose or starch and butter or margarine§ for 6 months*

(Mean values and standard deviations for eight rats/group)

		16:0		16:1		18:0		18:1		18:2ω^6		20:4ω^6		16:0:16:1		18:0:18:1	
Liver	Group	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
	Sucrose + butter	266	46	16	7	380	38	164	29	67	25	187	37	21·9	16·2	2·4	0·8
	Sucrose + margarine	242	39	trace		452	36	41	17	100	15	154	33	—		13·8	7·4
	Starch + butter	276	26	12	4	398	44	111	27	86	28	112	46	26·4	10·8	3·8	1·2
	Starch + margarine	228	33	trace		476	54	29	13	103	35	152	55	—		18·8	7·2
Analysis of variance		†				†††		*†††	‡	†						†††	
Plasma	Sucrose + butter	352	34	trace		349	28	206	32	50	16	28	18	—		1·7	0·4
	Sucrose + margarine	336	36	trace		489	80	42	22	74	23	55	22	—		16·7	11·8
	Starch + butter	347	39	trace		376	32	135	15	85	28	48	26	—		2·8	0·5
	Starch + margarine	337	34	trace		488	37	39	14	81	30	52	21	—		15·4	10·2
Analysis of variance						†††		***†††		*						††	

Differences between values for sucrose and starch groups: * $P < 0.05$, ***$P < 0.001$.
Differences between values for butter and margarine groups: † $P < 0.05$, †† $P < 0.01$, ††† $P < 0.001$.
Interaction between dietary carbohydrates and fats: ‡ $P < 0.05$.
§ For details of diets, see p. 240.

Table 6. *Composition of fatty acids (mmol/mol) in liver and plasma triglycerides of rats given diets containing sucrose or starch and butter or margarine§ for 6 months*

(Mean values and standard deviations for eight rats/group)

		14:0		16:0		16:1		18:0		18:1		18:2ω^6		16:0:16:1		18:0:18:1	
Liver	Group	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
	Sucrose + butter	14	4	379	19	46	19	27	5	519	28	14	4	9·8	4·8	0·05	0·01
	Sucrose + margarine	trace		457	49	19	12	42	23	281	27	196	70	44·9	23·4	0·16	0·10
	Starch + butter	14	3	406	27	38	22	34	11	457	18	51	21	14·0	8·0	0·07	0·03
	Starch + margarine	trace		431	61	5	5	41	25	217	31	307	88	138·0	62·0	0·19	0·10
Analysis of variance				††		†††				†‡		**†††		*†††‡		†††	
Plasma	Sucrose + butter	25	11	397	107	37	13	69	34	439	109	32	19	12·3	7·3	0·05	0·01
	Sucrose + margarine	13	3	390	106	21	12	70	32	224	43	268	80	25·3	18·2	0·16	0·10
	Starch + butter	28	10	402	22	46	12	67	23	401	44	53	9	9·7	4·5	0·07	0·03
	Starch + margarine	20	6	394	53	28	9	92	17	209	29	253	48	16·0	2·2	0·19	0·10
Analysis of variance		†††				†††				†††		†††		†††		†††	

Differences between values for sucrose and starch groups: * $P < 0·05$, ** $P < 0·001$.
Differences between values for butter and margarine groups: † $P < 0·05$, †† $P < 0·01$, ††† $P < 0·001$.
Interaction between dietary carbohydrate and fats: ‡ $P < 0·05$.
§ For details of diets, see p. 240.

sunflower oil (Wahle & Radcliffe, 1977). In earlier studies, fructose was found to reduce the linoleic acid content of the lipids (Macdonald *et al.* 1975). The different results could have been due to differences in the compositions of the diets used in the two laboratories.

Long-term feeding on sucrose-rich diets, especially when accompanied by butter, led to a lower proportion of linoleic acid in tissue lipids compared to those fed starch; this has been noted in other animals and human subjects (Macdonald & Braithwaite, 1964; Alling *et al.* 1973), but without a major change in the values for saturated:monounsaturated fatty acids. It is possible that the presence of large excesses of linoleic acid in the polyunsaturated margarine may mask, in some lipid fractions, the effect of sucrose that occurs when the supply of linoleic acid is more limited, as shown in experiments in which diets low in fats have been used (Bruckdorfer, unpublished results). The reduction in the percentage of linoleic acid may be the consequence of an increase in the hepatic synthesis of saturated and monounsaturated fatty acids in animals fed on sucrose or alternatively be an increase in linoleic acid oxidation. Increased endogenous synthesis of fatty acids was also thought to be the cause of the lower percentage of linoleic acid in the livers of obese Zucker rats compared to those found in their lean littermates (Wahle, 1974*b*). The presence of large amounts of linoleic acid such as those provided in the margarine diet is thought to depress both fatty acid synthetase and stearoyl CoA desaturase activities (Jeffcoat & James, 1977). The amount of arachidonate in the phospholipids was the same in animals fed on starch or sucrose; on the other hand, it was greater in those fed on margarine than in those fed on butter.

Although the linoleic acid content of maize starch is greater than that of sucrose, its contribution to the total diet is unlikely to have been enough to account for these effects of sucrose. In none of these tissues did significant quantities of trienoic acids arise to indicate an essential fatty acid deficiency. These findings may be of significance in human diets which are rich in both sucrose and animal fat, for which a synergistic action on lipid metabolism has been suggested (Antar *et al.* 1970).

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