The regulation of hepatic stearoyl-coenzyme A desaturase in obese–hyperglycaemic (*ob/ob*) mice by food intake and the fatty acid composition of the diet

Michael ENSER and Jennifer L. ROBERTS

Muscle Biology Division, Agricultural Research Council Meat Research Institute, Langford,
Bristol BS18 7DY, U.K.

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1. The effects of food intake and the fatty acid composition of the diet on the hepatic stearoyl-CoA desaturase activity of obese–hyperglycaemic (*ob/ob*) mice were investigated. 2. Obese mice fed on a commercial mouse diet, *ad libitum*, had 6.5-fold more activity per liver cell than had lean mice. 3. On a diet containing 14% corn oil the activity was 65% less in obese mice and 62% less in lean mice compared with animals fed on the commercial diet. 4. Feeding with 14% saturated fat in the diet doubled the activity in lean mice compared with those on the commercial diet, but had no effect on the activity in obese mice. 5. Obese mice fed on the corn-oil diet contained a higher proportion of linoleic acid in the liver lipids than did lean mice fed on the commercial diet, but the acyl-CoA desaturase activity was 125% higher than in the lean mice. 6. Limiting the food intake of obese mice by pair-feeding with lean mice decreased their acyl-CoA desaturase activity when the animals were fed on the saturated-fat diet, but the activity remained 75% higher than in lean mice, whereas in obese mice pair-fed on the corn-oil diet the activity was the same as in lean mice. 7. During starvation the acyl-CoA desaturase activity in livers from obese mice decreased more slowly and proportionately less than in livers from lean mice. 8. It is concluded that increased substrate supply as a result of hyperphagia and not low concentration of linoleic acid is the main factor causing high acyl-CoA desaturase activity in obese mice.

The proportions of palmitoleic acid and oleic acid in the liver and adipose tissue from obese–hyperglycaemic (*ob/ob*) mice are higher than in lean mice as a result of increased synthesis (Winand *et al.*, 1968, 1969*a,b*, 1973) by the microsomal acyl-CoA desaturase enzyme (Enser, 1975), but the factors responsible for this increased activity are only partially understood. Although the fat content of standard mouse diets is low, the saturated fatty acids, which are substrates for the enzyme as CoA derivatives, are probably readily available because of the high rate of fatty acid synthesis *de novo* in obese mice (Bates *et al.*, 1955*a,b*; Mayer *et al.*, 1955; Shigeta & Shreeve, 1964; Jansen *et al.*, 1967; Shreeve *et al.*, 1967). High concentrations of saturated fatty acids in the diet increase acyl-CoA Δ^9 -desaturase activity in fed rats (Inkpen *et al.*, 1969) and diabetic rats (Mercuri *et al.*, 1974), and it seems likely that similar action by endogenously synthesized saturated fatty acids could account for the increased desaturase activity in obese mice. In support of this, a close relationship has been

reported between fatty acid synthesis *de novo* and acyl-CoA Δ^9 -desaturase activity (Wahle & Garton, 1972; Wahle, 1974; Jeffcoat & James, 1977; Jeffcoat *et al.*, 1979).

The hyperinsulinaemia of obese mice (Christophe *et al.*, 1959; Stauffacher *et al.*, 1967) has been suggested as the cause of the high rates of fatty acid synthesis (Assimakopoulos-Jeannet *et al.*, 1974; Loten *et al.*, 1976). Insulin has also been implicated in the regulation of hepatic acyl-CoA Δ^9 -desaturase because it restores to normal the low activity of diabetic rats (Gellhorn & Benjamin, 1964) and increases the activity in normal rats (Inkpen *et al.*, 1969). However, when the concentration of plasma insulin in obese mice was decreased to that in lean mice by alloxan treatment, the activity of the hepatic acyl-CoA desaturase was not decreased (Enser, 1979). The alloxan-treated mice retained the hyperphagia that is characteristic of the *ob* mutant, and this may have been responsible, in part, for the retention of a high acyl-CoA desaturase activity, but dietary restriction alone was only partially effective

in lowering the desaturase activity, since obese mice given half as much food as lean mice maintained twice the hepatic acyl-CoA desaturase activity (Enser, 1979).

Obese mice fed on a standard mouse diet have low proportions of polyunsaturated fatty acids in their liver lipids compared with lean mice (Haessler & Crawford, 1965; Stein *et al.*, 1967; Winand *et al.*, 1969b; Bulfield, 1972), and dietary polyunsaturated fatty acids have been demonstrated to regulate acyl-CoA desaturase activity (Inkpen *et al.*, 1969; Holloway & Holloway, 1975; Jeffcoat & James, 1977, 1978; Jeffcoat *et al.*, 1979). Genetically obese (Zucker) (*fa/fa*) rats also have high hepatic acyl-CoA desaturase activity, which can be decreased to that in lean rats by feeding them on a diet rich in linoleic acid (Wahle & Radcliffe, 1977). We have previously reported (Enser, 1979) a good inverse correlation between the proportion of linoleic acid in the liver lipids and hepatic acyl-CoA desaturase activity across several treatment groups of lean and obese mice, which was fitted by a common regression line. However, under none of our conditions was the activity in tissues from obese mice decreased to that of lean mice.

To evaluate the significance of food intake and linoleic acid concentration in producing the elevated acyl-CoA desaturase activity of obese mice, we have determined the hepatic acyl-CoA desaturase activity in lean and obese mice fed on diets containing saturated or polyunsaturated fatty acids under conditions of feeding *ad libitum* or in obese mice restricted to the same food intake as lean mice. We have analysed the fatty acid composition of the liver lipids to determine whether the inverse relationship between the linoleic acid content and acyl-CoA desaturase activity that we previously observed was maintained under these conditions, which involve a wider range of linoleic acid concentrations. Finally we have investigated the effect of complete withdrawal of exogenous substrate fatty acids and precursors on hepatic acyl-CoA desaturase activity by starving the mice, since previous studies have shown other enzymes of lipid metabolism in obese mice to be refractory to this treatment (Enser, 1972; Maragoudakis *et al.*, 1974; Volpe & Marasa, 1975).

Materials and methods

Experimental procedure

The animals and procedures were as described previously (Enser, 1972, 1975, 1979), except as follows. Female obese (*ob/ob*) and lean (+/+) mice of the C57B1/6J strain bred in our own colony were used. They were kept under controlled illumination of 12h light/12h dark, and drinking water was always available. When weaned, mice were fed on a

commercial pelleted diet (Oxoid breeding diet), which is the standard diet in the experiments.

The composition of the experimental diets was similar to a balanced diet used by De Gomez Dumm *et al.* (1970). Each consisted of (w/w) 51% maize starch, 24% casein, 3% cellulose, 4% salt mixture, 2% vitamin mixture, 2% dried baker's yeast and either 14% hydrogenated coconut oil or 14% corn oil. The ingredients were mixed to a dough with water, cut into cubes and freeze-dried to obtain an easily handled form. The experimental diets contained 74.8 kJ of gross energy per g of protein, assuming that the energy values of protein, fat and carbohydrate are 22.0, 39.6 and 17.6 kJ/g respectively. The metabolizability was assumed to be 0.91. The standard diet contains approx. 70 kJ/g of protein and 11.87 MJ of metabolizable energy/kg (manufacturers' quoted value). To extract the fat, portions of the diets were ground into powder and refluxed for at least 3 h with diethyl ether in a Soxhlet extraction apparatus. The preparation of fatty acids and analysis by g.l.c. was as described previously (Enser, 1975).

Mice were fed on the experimental diets from 6 to 12 weeks of age. For measurements of food intake *ad libitum*, mice were housed in groups of two to four, obese or lean, and residual food, including that spilt, was weighed every 2–3 days. The intake per mouse was calculated from these measurements and the value shown in Table 2 is the average metabolizable energy intake over the 11 days before the animals were killed. Pair-feeding started at 10 weeks of age with obese mice that had eaten the diets *ad libitum* from the age of 6 weeks. The quantity eaten by a minimum of three lean mice, housed together, was measured each day at 11:00h. The average amount eaten per mouse was calculated and this quantity was fed to obese mice of the same age, in two portions, one at 17:00h the same day and one at 08:30h the following day. These obese mice were housed singly, and each portion of food was placed on the metal grid that forms the roof of the cage, to prevent the mice from eating quickly. For the starvation studies 12-week-old mice were placed in clean cages, with drinking water but without food, at 11:00h, and were killed after 24, 48 or 72 h between 11:00 and 12:00h.

Mice were anaesthetized with diethyl ether and blood was taken from the retrobulbar sinus, for preparation of plasma for insulin determination. The mice were then killed by decapitation, the liver was immediately removed and the acyl-CoA desaturase activity was determined by measuring the conversion of [14 C]stearic acid into [14 C]oleic acid by the previously described protocol (Enser, 1975).

Chemicals

Analytical-grade reagents were used if possible.

and solvents were purified by distillation. Maize starch and casein were obtained from BDH Chemicals (Poole, Dorset, U.K.). Cellulose was from Reeve Angel (London E.C.4, U.K.). Salt mixture XIV, vitamin diet fortification mixture and hydrogenated coconut oil were from ICN Pharmaceuticals (Cleveland, OH, U.S.A.). Baker's yeast was a retail preparation for home baking (Distillers Co.), and pure corn oil was from Boots Co. (Nottingham, U.K.).

Statistics

Significant differences were identified by analysis of variance and Student's *t* test. *P* values of <0.05 were considered significant. Simple linear-regression analysis was used.

Results

The experimental diets contained 14% fat, whereas the standard pelleted diet contained only 3.1% fat. The fatty acid composition of the fat extracted from the standard diet included 18% of palmitic acid and stearic acid, which are the major substrates for the acyl-CoA Δ^9 -desaturase (Marsh & James, 1962; Gellhorn & Benjamin, 1964), and 43.2% linoleic acid, which is known to regulate the enzyme activity (Inkpen *et al.*, 1969; Jeffcoat & James, 1978) (Table 1). The fat extracted from the corn-oil diet had a similar fatty acid composition to that from the standard diet except that the proportion of palmitic acid was less (11.0% compared with 15.3%) and that of linoleic acid was greater (58.7%). The fatty acids in the hydrogenated-coconut-oil diet

were mainly saturated and included 27.9% of palmitic acid and stearic acid and large proportions of medium-chain fatty acids. Linoleic acid was not detected.

Both obese and lean mice ate less, *ad libitum*, of the experimental diets than of standard diet (Table 2), but, because of the higher caloric density of these diets, their metabolizable energy intake was unchanged. The average amount of linoleic acid consumed daily was calculated to be 252 mg for obese mice and 163 mg for lean mice with corn-oil diet *ad libitum*, whereas with standard diet it was 55 mg and 37 mg respectively.

Growth rates of obese and lean mice eating the experimental diets *ad libitum* were similar to those obtained with the standard diet except for obese mice fed on the corn-oil diet, which were 10% heavier after 6 weeks on the diet (Table 2). Obese mice that began pair-feeding, after 4 weeks of feeding *ad libitum* on the same diet, initially lost weight. Mice weighed 5% less with the hydrogenated coconut-oil diet after 3 days of pair-feeding, but had regained the weight after a further 3–4 days. Pair-feeding with the corn-oil diet caused a 4% loss in weight after 3 days, and this was regained more slowly over the remaining 8 days. Pair-fed obese mice weighed 14.5% and 15.9% less when they were killed than did mice fed *ad libitum*, for the hydrogenated coconut-oil and corn-oil diets respectively.

Stearoyl-CoA desaturase activity in mice fed *ad libitum*

Obese mice fed on the standard diet showed the characteristic elevation of stearoyl-CoA desaturase activity, compared with lean mice (Table 3). Total activity was 11-fold greater in obese mice because of increased liver weight, and also a 6.5-fold increase in activity per mg of DNA. Lean mice fed on the hydrogenated-coconut-oil diet had 2.5-fold greater total acyl-CoA desaturase activity in the liver than did those fed on the standard diet, whereas the total activity in livers of obese mice was the same with both diets (Table 3). However, the hydrogenated-coconut-oil diet resulted in 24–25% more hepatic DNA in both obese and lean mice (Table 3), so that obese mice had 25% less acyl-CoA desaturase activity on a cellular basis than did those fed on the standard diet, and lean mice had a doubled activity per cell. Therefore obese mice had only 3.5-fold more activity per liver cell than lean mice when they were fed on the hydrogenated-coconut-oil diet. The response to the corn-oil diet was similar in obese mice to that in lean mice, in that the total liver acyl-CoA desaturase activities were lower by 65% and 62% respectively compared with values obtained with the standard diet (Table 3). The DNA content of the liver was unchanged with the corn-oil

Table 1. *Fatty acid composition of diets*

Standard diet was Oxoid breeding diet for rats and mice, containing 3.1% fat. Hydrogenated-coconut-oil diet and corn-oil diet were semi-synthetic diets containing 14% added oil. For full details see the Materials and methods section.

Fatty acid	Fatty acid composition (% by wt.)		
	Standard diet	Hydrogenated-coconut-oil diet	Corn-oil diet
C _{10:0}		6.8	
C _{12:0}		39.2	
C _{14:0}	1.0	19.5	0.1
C _{16:0}	15.3	11.6	11.0
C _{16:1}	1.1	0.2	0.3
C _{18:0}	2.9	16.3	2.0
C _{18:1}	26.1	5.2	25.8
C _{18:2}	43.2		58.7
C _{18:3}	2.3		1.1
C _{20:0}		0.1	
Others	8.1	1.1	1.0

Table 2. *Effects of dietary lipid on body weight, food intake and plasma insulin of obese and lean mice fed ad libitum and pair-fed obese mice*

For experimental details see the text. Mice were fed *ad libitum*, unless indicated, on standard laboratory diet or diet containing hydrogenated coconut oil (HCO) or corn oil. Results are means $\pm$ s.e.m. for at least 15 mice in each group. Numbers within columns with different superscript letters differ significantly at $P < 0.05$.

Type of mouse	Diet	Body weight (g)		Metabolizable energy intake (kJ/day)	Concn. of plasma insulin (μ units/ml)
		Initial	Final		
Obese	Standard	22.6 $\pm$ 0.9 ^c	43.4 $\pm$ 0.7 ^e	54.5	1052 $\pm$ 137 ^{b,c}
Lean	Standard	15.8 $\pm$ 0.4 ^a	20.1 $\pm$ 0.2 ^a	36.8	40.6 $\pm$ 4.2 ^a
Obese	HCO	23.7 $\pm$ 1.0 ^c	43.7 $\pm$ 0.7 ^e	55.5	846 $\pm$ 68 ^b
Obese	HCO, pair-fed	22.3 $\pm$ 1.3 ^c	37.4 $\pm$ 0.9 ^c	34.3	1498 $\pm$ 206 ^c
Lean	HCO	17.5 $\pm$ 0.3 ^b	21.1 $\pm$ 0.3 ^b	34.3	62.3 $\pm$ 9.2 ^a
Obese	Corn oil	23.2 $\pm$ 1.1 ^c	47.8 $\pm$ 0.7 ^f	55.5	1113 $\pm$ 110 ^{b,c}
Obese	Corn oil, pair-fed	24.3 $\pm$ 0.8 ^c	40.2 $\pm$ 0.6 ^d	35.9	2437 $\pm$ 289 ^d
Lean	Corn oil	16.5 $\pm$ 0.3 ^a	20.6 $\pm$ 0.3 ^{a,b}	35.9	46.5 $\pm$ 4.9 ^a

Table 3. *Effects of dietary lipid on hepatic stearic acid desaturase activity of obese and lean mice fed ad libitum and pair-fed obese mice*

For experimental details see the text. Mice were fed *ad libitum*, unless indicated, on standard laboratory diet or diet containing hydrogenated coconut oil (HCO) or corn oil. Results are means $\pm$ s.e.m. for at least 15 mice in each group. Numbers within columns with different superscript letters differ significantly at $P < 0.05$.

Type of mouse	Diet	Oleate formed		Liver weight (g)	DNA (mg/liver)
		(μ mol/h per liver)	(μ mol/h per mg of DNA)		
Obese	Standard	31.82 $\pm$ 1.71 ^f	5.63 $\pm$ 0.37 ^f	3.39 $\pm$ 0.07 ^f	5.75 $\pm$ 0.37 ^d
Lean	Standard	2.78 $\pm$ 0.31 ^b	0.85 $\pm$ 0.09 ^b	0.97 $\pm$ 0.01 ^a	3.30 $\pm$ 0.09 ^a
Obese	HCO	30.15 $\pm$ 2.29 ^f	4.25 $\pm$ 0.26 ^e	3.44 $\pm$ 0.18 ^f	7.13 $\pm$ 0.26 ^e
Obese	HCO, pair-fed	13.83 $\pm$ 0.63 ^e	2.97 $\pm$ 0.20 ^d	2.18 $\pm$ 0.04 ^d	4.78 $\pm$ 0.20 ^c
Lean	HCO	6.75 $\pm$ 0.43 ^c	1.68 $\pm$ 0.11 ^c	1.14 $\pm$ 0.03 ^b	4.09 $\pm$ 0.11 ^b
Obese	Corn oil	10.79 $\pm$ 0.60 ^d	1.93 $\pm$ 0.11 ^c	2.54 $\pm$ 0.08 ^e	5.66 $\pm$ 0.11 ^d
Obese	Corn oil, pair-fed	2.01 $\pm$ 0.30 ^b	0.43 $\pm$ 0.06 ^{a,b}	1.65 $\pm$ 0.04 ^c	4.66 $\pm$ 0.06 ^d
Lean	Corn oil	1.15 $\pm$ 0.19 ^a	0.33 $\pm$ 0.06 ^a	1.01 $\pm$ 0.02 ^a	3.60 $\pm$ 0.06 ^a

diet, although the livers from the obese mice weighed 25% less than those from obese mice fed on the standard diet (Table 3). When eating the corn-oil diet obese mice had 6-fold greater acyl-CoA desaturase activity per cell than lean mice, a ratio similar to that observed for the standard diet.

Stearoyl-CoA desaturase activity in pair-fed obese mice

The hepatic stearoyl-CoA desaturase activity of obese mice pair-fed on experimental diets with lean mice was lower than that of obese mice fed on the same diets *ad libitum* (Table 3). Pair-fed obese mice had the same acyl-CoA desaturase activity when expressed per g wet wt. of tissue as the lean mice whose food intake determined their ration. The livers were two-thirds of the weight of those from obese mice fed on the same diet *ad libitum*, but remained heavier than those from lean mice and therefore had a greater total acyl-CoA desaturase activity (Table

3). The hepatic DNA content of obese mice was less with pair-feeding, by 33% with the hydrogenated-coconut-oil diet and by 17% with the corn-oil diet (Table 3). Obese mice pair-fed on the hydrogenated-coconut-oil diet had 30% less activity per cell than did obese mice fed on the same diet *ad libitum*, but significantly ($P < 0.001$) more than lean mice. However, with the corn-oil diet, pair-fed obese mice had 78% less activity per cell than did obese mice fed *ad libitum*. This was half of that in lean mice fed on the standard diet and was not significantly different ($P > 0.05$) from that of the lean mice fed on the corn-oil diet.

Effect of starvation on hepatic stearoyl-CoA desaturase activity

Starved lean mice had markedly lower acyl-CoA desaturase activity than did fed lean mice (Table 4). After 24 h starvation the activity was 15% of that in fed mice, and only 1.7% was measured in 72 h-

starved mice. The effect of starvation was less marked in obese mice, with 52% after 24 h and 10% after 72 h of the total liver activity compared with fed mice (Table 4). The acyl-CoA desaturase activity per cell of 72 h-starved obese mice was the same as that of fed lean mice and was over 70-fold greater than that of 72 h-starved lean mice. Starved lean mice had similar amounts of hepatic DNA to fed lean mice, but after 72 h starvation their livers weighed 21% less. Livers from obese mice starved for 72 h contained 19% less DNA and weighed 46% less than those of fed obese mice (Table 4). Starvation of 72 h brought about a loss in weight of 23% for lean mice and of 13.6% for obese mice (Table 4).

Hepatic lipid content and fatty acid composition

The livers from obese mice fed on the standard diet *ad libitum* contained approx. 17% fat compared with 5% in the livers from lean mice, and similar proportions were found for mice fed on the corn-oil

diet (Tables 3 and 5). The hydrogenated-coconut-oil diet fed *ad libitum* resulted in a greater proportion of fat stored in the liver, namely 21% and 8.5% of the wet tissue weight for obese and lean mice respectively, which is explained by the influx of medium-chain fatty acids from the intestine via the portal vein. The livers from pair-fed obese mice, besides being smaller than those from obese mice fed *ad libitum*, contained lower proportions of fat, at 17% with the hydrogenated-coconut-oil diet and 8.8% with the corn-oil diet.

The fatty acid composition of the liver lipids was examined to determine its relationship to the acyl-CoA Δ^9 -desaturase activity and the dietary fatty acids (Table 5). The livers from obese mice fed on the standard diet displayed the typically doubled proportions of the products of Δ^9 -desaturation, palmitoleic acid and oleic acid, compared with livers from lean mice. But, whereas similar proportions of palmitic acid were present, livers from obese mice had one-fifth the proportion of stearic acid found in the lean mice, perhaps reflecting the substrate-

Table 4. *Effects of starvation on hepatic stearoyl-CoA desaturase activity of obese and lean mice*
For experimental details see the text. Results are means $\pm$ S.E.M. for nine female mice in each group.

	Type of mouse	Period of starvation	...	0 h	24 h	48 h	72 h
Oleate formed ($\mu\text{mol/h}$ per liver)	Obese			35.59 $\pm$ 2.80	18.42 $\pm$ 1.86	4.97 $\pm$ 0.68	3.59 $\pm$ 0.85
	Lean			3.54 $\pm$ 0.36	0.55 $\pm$ 0.18	0.06 $\pm$ 0.05	0.04 $\pm$ 0.01
Weight of liver (g)	Obese			3.37 $\pm$ 0.12	2.29 $\pm$ 0.10	2.02 $\pm$ 0.07	1.82 $\pm$ 0.08
	Lean			1.04 $\pm$ 0.07	0.76 $\pm$ 0.02	0.76 $\pm$ 0.04	0.82 $\pm$ 0.02
DNA (mg/liver)	Obese			6.09 $\pm$ 0.42	6.11 $\pm$ 0.39	5.60 $\pm$ 0.39	4.91 $\pm$ 0.14
	Lean			3.75 $\pm$ 0.23	3.41 $\pm$ 0.27	3.49 $\pm$ 0.28	3.57 $\pm$ 0.14
Weight of mice when killed (g)	Obese			42.38 $\pm$ 0.81	39.04 $\pm$ 0.70	37.65 $\pm$ 0.43	35.86 $\pm$ 0.89
	Lean			21.81 $\pm$ 0.91	18.36 $\pm$ 0.46	16.67 $\pm$ 0.19	16.96 $\pm$ 0.28
Weight loss (g)	Obese				3.60 $\pm$ 0.11	4.83 $\pm$ 0.19	5.62 $\pm$ 0.35
	Lean				2.58 $\pm$ 0.08	3.89 $\pm$ 0.09	5.06 $\pm$ 0.19

Table 5. *Effects of dietary lipid on fat content and fatty acid composition of liver lipids from obese and lean mice fed ad libitum and pair-fed obese mice*

For experimental details see the text. Mice were fed *ad libitum*, unless indicated, on standard laboratory diet or diet containing hydrogenated coconut oil (HCO) or corn oil. Results are means $\pm$ S.E.M. for at least 15 mice in each group. Numbers within columns with different superscript letters differ significantly at $P < 0.05$.

Type of mouse	Diet	Fat content (mg/liver)	Fatty acid composition (% by wt.)						
			C _{14:0}	C _{16:0}	C _{16:1}	C _{18:0}	C _{18:1}	C _{18:2}	C _{20:4}
Obese	Standard	572.4 $\pm$ 29.8 ^e	0.5 ^c	22.7 ^{d,e}	8.4 ^f	2.4 ^b	57.5 ^f	3.2 ^d	1.3 ^b
Lean	Standard	52.5 $\pm$ 3.7 ^d	0.4 ^b	24.3 ^e	3.2 ^b	11.7 ^f	25.6 ^b	13.6 ^e	10.0 ^f
Obese	HCO	743.3 $\pm$ 64.4 ^f	1.1 ^d	21.4 ^{b,c,d}	7.0 ^e	2.0 ^a	61.9 ^g	1.0 ^a	0.7 ^a
Obese	HCO, pair-fed	368.3 $\pm$ 15.8 ^d	1.6 ^e	20.5 ^{a,b,c}	9.0 ^g	3.0 ^c	56.2 ^f	2.1 ^b	1.6 ^c
Lean	HCO	97.1 $\pm$ 6.8 ^b	2.1 ^f	19.9 ^{a,b}	5.7 ^d	5.9 ^d	50.4 ^e	2.6 ^c	3.7 ^d
Obese	Corn oil	403.2 $\pm$ 27.0 ^d	0.5 ^c	21.3 ^{b,c}	5.8 ^d	3.3 ^c	44.5 ^d	16.3 ^f	3.6 ^d
Obese	Corn oil, pair-fed	145.6 $\pm$ 8.3 ^c	0.5 ^c	22.3 ^{c,d,e}	4.3 ^c	7.6 ^e	28.7 ^c	23.4 ^g	6.5 ^e
Lean	Corn oil	58.0 $\pm$ 3.2 ^a	0.2 ^a	18.5 ^a	1.7 ^a	11.4 ^f	19.5 ^a	28.6 ^h	11.6 ^g

specificity of the enzyme (Paulsrud *et al.*, 1970). The proportions of polyunsaturated fatty acids were less than one-quarter of those of livers from lean mice. Liver lipids of obese mice fed on the hydrogenated-coconut-oil diet *ad libitum* displayed a similar fatty acid composition to that of obese mice fed on the standard diet, but the proportions of polyunsaturated fatty acids were less, with only 1% linoleic acid, and more medium-chain fatty acids were present. The fatty acid composition of liver lipids of lean mice fed on the hydrogenated-coconut-oil diet also resembled this pattern, although the proportions of oleic acid and palmitoleic acid were not as high and the proportion of stearic acid was not as low as in obese mice fed on either this diet or the standard diet. The proportion of linoleic acid in the livers of lean mice fed on the hydrogenated-coconut-oil diet was not significantly different from that of obese mice fed on the standard diet. The high linoleic acid content of the corn-oil diet was clearly reflected in the fatty acid composition of the liver lipids (Table 5). Obese mice fed *ad libitum* had 5-fold more linoleic acid and 3-fold more arachidonic acid, and lean mice had double the proportion of linoleic acid, compared with those mice fed on the standard diet. The proportions of monounsaturated fatty acids were less in the liver lipids of both obese and lean mice fed on the corn-oil diet *ad libitum*.

Pair-feeding obese mice on either of the experimental diets resulted in proportions of fatty acids in the liver lipids that were, in general, intermediate between those of obese and lean mice fed on the same diet *ad libitum* (Table 5). With the hydrogenated-coconut-oil diet the proportions of fatty acids were most similar to those of obese mice fed on the standard diet *ad libitum*. Obese mice pair-fed on the corn-oil diet had similar proportions of monounsaturated fatty acids to those of lean mice fed on the standard diet, but the proportions of poly-

unsaturated fatty acids were lower except for that of linoleic acid, which was nearly twice as high.

The livers from lean mice starved for 24 h contained twice as much lipid as did livers from fed mice, whereas starved obese mice had less liver fat than did fed obese mice (Table 6). In livers of starved lean mice the proportions of oleic acid and linoleic acid were significantly increased and the proportions of stearic acid and arachidonic acid were decreased. The 24 h-starved lean mice also had significantly increased palmitoleic acid, but this difference was not found in the lean mice starved for longer periods (Table 6). Significantly lower proportions of monounsaturated fatty acids and 2–3-fold higher proportions of polyunsaturated fatty acids were present in livers of obese mice starved for 72 h than in those of fed obese mice. The 24 h-starved and 48 h-starved obese mice had intermediate proportions of these fatty acids.

Effect of diets on plasma insulin concentration

The concentrations of insulin in plasma from lean mice fed on the experimental diets were not significantly different from those of lean mice fed on the standard diet (Table 2). All obese mice were hyperinsulinaemic regardless of type of diet or quantity eaten (Table 2). The composition of the diet did not affect the concentration of plasma insulin in obese mice eating *ad libitum*, but for each diet pair-fed obese mice had significantly higher concentrations of plasma insulin than did those eating *ad libitum*.

Discussion

The present study demonstrates that the hyperphagia in obese mice is a major factor causing the high activity of hepatic stearyl-CoA desaturase compared with lean mice. Obese mice fed *ad libitum*

Table 6. *Effects of starvation on fat content and fatty acid composition of liver lipids from obese and lean mice*
For experimental details see the text. Results are means $\pm$ S.E.M. for nine female mice in each group fed on standard laboratory diet before starvation. Numbers within columns with different superscript letters differ significantly at $P < 0.05$.

Type of mouse	Period of starvation (h)	Fat content (mg/liver)	Fatty acid composition (% by wt.)					
			C _{16:0}	C _{16:1}	C _{18:0}	C _{18:1}	C _{18:2}	C _{20:4}
Obese	0	504.9 $\pm$ 25.8 ^f	21.5 ^{a,b}	8.3 ^e	2.6 ^a	57.6 ^e	3.9 ^a	1.3 ^a
Lean	0	38.8 $\pm$ 3.7 ^a	23.6 ^{b,c}	4.0 ^b	8.4 ^e	25.4 ^a	15.5 ^e	8.8 ^e
Obese	24	329.6 $\pm$ 20.1 ^c	22.4 ^{a,b}	7.2 ^d	2.7 ^a	54.0 ^d	5.8 ^b	2.1 ^{a,b}
Lean	24	67.8 $\pm$ 6.6 ^b	25.1 ^c	4.7 ^c	4.7 ^c	28.4 ^{a,b}	21.1 ^f	4.8 ^d
Obese	48	336.2 $\pm$ 26.3 ^e	21.6 ^{a,b}	7.0 ^d	3.3 ^b	52.4 ^d	8.4 ^c	2.3 ^{a,b}
Lean	48	88.6 $\pm$ 11.7 ^c	23.3 ^{b,c}	3.3 ^a	4.9 ^c	29.6 ^b	23.9 ^e	4.2 ^{c,d}
Obese	72	261.3 $\pm$ 16.7 ^d	20.7 ^a	6.9 ^d	3.6 ^b	48.0 ^c	10.3 ^d	3.2 ^{b,c}
Lean	72	71.5 $\pm$ 6.7 ^{b,c}	20.8 ^a	3.2 ^a	6.4 ^d	29.8 ^b	22.8 ^e	4.7 ^d

ate more than lean mice did and had higher hepatic acyl-CoA desaturase activities regardless of the fatty acid composition of the diet. When obese mice were fed on the quantity of food consumed by lean mice, the acyl-CoA desaturase activity was lower to an extent that depended on the amount and type of fatty acids in the diet. However, hyperphagia and the type of dietary fat alone cannot explain all the differences in acyl-CoA desaturase activity between obese and lean mice, since during starvation for 72 h obese mice lost 89% of their total hepatic activity, whereas lean mice lost 99%.

The action of food in increasing acyl-CoA desaturase activity appears to depend on the provision of substrate fatty acids in the diet or through synthesis *de novo*, since both glucose and hydrogenated coconut oil restore the desaturase activity in starved rats (Inkpen *et al.*, 1969). However, the supply of substrate in rats fed on a standard pelleted diet was insufficient to induce the enzyme maximally, since the activity was raised by including saturated fatty acids in the diet (Mercuri *et al.*, 1974; Jeffcoat & James, 1977). In the present study lean mice fed on the hydrogenated-coconut-oil diet responded like rats and had twice the acyl-CoA desaturase activity of mice fed on the standard diet. On the other hand obese mice did not have higher activities when fed on the hydrogenated-coconut-oil diet *ad libitum*, possibly because the high rate of fatty acid synthesis of these mice (Bates *et al.*, 1955a,b; Mayer *et al.*, 1955; Shigeta & Shreeve, 1964; Jansen *et al.*, 1967; Shreeve *et al.*, 1967) provides sufficient substrate to induce the enzyme maximally. However, the acyl-CoA desaturase activity of the obese mouse retains a response to substrate supply, as shown by the lower activity that results from pair-feeding. The enzyme activity responded to dietary corn oil with the expected decrease, indicating sensitivity to the repressive action of linoleic acid (Inkpen *et al.*, 1969). The proportion of linoleic acid in the liver lipids was lower in the obese mice fed on the hydrogenated-coconut-oil diet *ad libitum* than in those fed on the standard diet (Table 5), so it is unlikely that linoleic acid was preventing the saturated fatty acids from increasing the enzyme activity.

The regulation of stearoyl-CoA desaturase by linoleic acid can occur through direct inhibition of the enzyme (Uchiyama *et al.*, 1967) or by an inhibition of enzyme synthesis (Inkpen *et al.*, 1969; Jeffcoat *et al.*, 1979). The effects of the corn-oil diet in the present study are the result of changes in the quantity or possibly the specific activity of the enzyme and not through inhibition by free linoleic acid in the enzyme preparation, since we have previously established that the concentration of endogenous non-esterified fatty acids is too low to compete with the substrate stearic acid (Enser,

1975). Both obese and lean mice responded to the corn-oil diet with lower acyl-CoA desaturase activities: 55% and 81% less respectively per liver cell compared with values obtained with the hydrogenated-coconut-oil diet. This difference in the extent of the response between obese and lean mice was the result of hyperphagia in the obese mice, and was removed by pair-feeding, when obese and lean mice had similar activities: $0.43 \mu\text{mol/h}$ per mg of DNA and $0.32 \mu\text{mol/h}$ per mg of DNA ($P > 0.05$) respectively. However, it is not clear whether hyperphagia as such or the proportion of linoleic acid in the liver lipids was the major regulator, since the obese mice fed on the corn-oil diet *ad libitum* had a significantly lower proportion of linoleic acid than did lean mice or obese mice pair-fed on the same diet. The effect of hyperphagia is more clearly demonstrated by comparison of obese mice fed on the corn-oil diet *ad libitum* with lean mice fed on the standard diet. The liver lipids have 20% more linoleic acid in the obese mice, yet the acyl-CoA desaturase activity is 125% higher than in the lean mice. Another similar comparison is between obese mice fed on the standard diet with 3.2% linoleic acid in the liver lipids and lean mice fed on the hydrogenated-coconut-oil diet with 2.6% linoleic acid in the liver lipids, where the obese mice have 3.4-fold more activity per cell (Table 5). The ineffectiveness of linoleic acid in overcoming the effect of hyperphagia is indicated by comparison of the lean mice fed on the hydrogenated-coconut-oil diet and obese mice fed on the corn-oil diet *ad libitum*. Their acyl-CoA desaturase activities did not differ significantly, but the proportion of linoleic acid in the liver lipids was 6.5-fold higher in obese mice.

The relationship between the proportion of linoleic acid in the liver lipids and the acyl-CoA desaturase activity (Fig. 1) is not uniform. Increases in the proportion of linoleic acid from 24% to 35% had little effect on the acyl-CoA desaturase activity, whereas below 5% linoleic acid large fluctuations in activity occurred. Since the mechanism by which linoleic acid regulates the acyl-CoA desaturase and the type of lipid involved are unknown, the absence of a direct relationship between the acyl-CoA desaturase activity and the proportion of linoleic acid in the liver lipids, over the whole range, is not unexpected. Changes in the composition or quantity of non-regulatory lipids may well mask changes in the specific effector. In an attempt to separate the effects of food intake from those of linoleic acid, we have analysed the relationship for lean mice and pair-fed mice in one group and obese mice feeding *ad libitum* in the other, but excluding the two groups with the highest concentrations of linoleic acid. The regression lines (Fig. 1) are almost parallel, suggesting normal sensitivity to linoleic acid but with a higher activity induced by hyperphagia. The uneven

Fig. 1. Relationship between the proportion of linoleic acid present in mouse hepatic lipids and the activity of stearyl-CoA desaturase

For experimental details see the text. ○, Obese mice fed on standard diet *ad libitum*; ●, lean mice, standard diet; □, obese mice fed on hydrogenated-coconut-oil diet *ad libitum*; ◇, obese mice pair-fed on hydrogenated-coconut-oil diet; ■, lean mice, hydrogenated-coconut-oil diet; △, obese mice fed on corn-oil diet *ad libitum*; ▽, obese mice pair-fed on corn-oil diet; ▲, lean mice, corn-oil diet. Regression equation $y = a + bx$. For obese mice fed *ad libitum* (○, □, △) $a = 105.255$, $b = -3.658$ and $r = -0.855$, $P < 0.001$. For lean mice fed on standard and hydrogenated-coconut-oil diets and obese mice pair-fed on the hydrogenated-coconut-oil diet (○, ■, ◇) $a = 69.504$, $b = 3.139$ and $r = -0.793$, $P < 0.001$.

distribution of data makes such a conclusion only tentative, although we have previously observed a linear response between 3% and 20% linoleic acid (Enser, 1979).

In fed mice the proportion of oleic acid in the liver lipids reflected the acyl-CoA desaturase activity but seemed to reach a limiting value of approx. 60% at activities greater than $3 \mu\text{mol}$ of oleate formed/h per mg of DNA. This high concentration occurred in obese mice fed on the standard diet or the hydrogenated-coconut-oil diet either *ad libitum* or by pair-feeding, despite large differences in the quantity of liver lipids produced by the different dietary regimes (Table 5). During starvation, although there were marked changes in acyl-CoA desaturase activity, the proportion of oleic acid was little changed, with a slight decrease in obese mice and

an increase in lean mice. The changes could be explained by mobilization of lipid from adipose tissue in which the proportions of oleic acid are respectively lower and higher than in the liver (Enser, 1979), but whereas the liver fat content increased by 84% in starved lean mice it decreased by 48% in starved obese mice.

The present data support our previous finding that insulin is not an important regulator of the elevated acyl-CoA desaturase activity in obese mice (Enser, 1979). Obese mice fed *ad libitum* had similar concentrations of plasma insulin on all diets, over which there was a 3-fold range in acyl-CoA desaturase activity (Tables 2 and 3). Also, pair-fed obese mice had lower acyl-CoA desaturase activities than did obese mice fed on the same diet *ad libitum* in the presence of higher concentrations of plasma

insulin. The high insulin concentration in mice pair-fed on the 14%-fat diets seems to be a characteristic of the fat content of the diet, and was unexpected, since restricting the intake of standard commercial diets decreased the concentration of plasma insulin in obese mice (Dubuc, 1976; Enser, 1979). Lemonnier *et al.* (1971) observed a decrease in plasma insulin concentration when obese mice were fed on a 40%-fat diet, but the low carbohydrate content of that diet, 10% compared with 51% in the present study, may have been the cause.

The decreased response to starvation of the acyl-CoA desaturase in obese mice compared with lean mice is similar to that previously reported for hepatic lipogenesis (Bates *et al.*, 1955a; Mayer *et al.*, 1955; Hems *et al.*, 1975), acetyl-CoA carboxylase (Maragoudakis *et al.*, 1974) and fatty acid synthetase activity (Volpe & Marasa, 1975). Thus maintenance of a relatively high acyl-CoA desaturase activity in starving obese mice may depend on the provision of substrate by an abnormally high synthesis of fatty acids *de novo*, as we have proposed for the fed state. Volpe & Marasa (1975) proposed that the slow decrease in the concentration of plasma insulin in starving obese mice could explain the delayed fall in fatty acid synthetase activity. However, although insulin concentrations in starved obese mice remain above those in fed lean mice, we have previously eliminated the possibility of these high concentrations acting as controlling factors in regulation of the acyl-CoA desaturase activity (Enser, 1979). The increase in the percentage of linoleic acid in the liver lipids during starvation was proportionately greater in obese than in lean mice (Table 6), which suggests that linoleic acid is not very significant in regulating acyl-CoA desaturase activity during starvation.

Overall, our results show that the regulation of acyl-CoA desaturase activity in obese mice is similar to that in lean mice and that the increased activity is the result of increased provision of substrate fatty acids. These substrate fatty acids are made available either in the diet or through increased lipogenesis *de novo* fuelled by hyperphagia. Increased synthesis of fatty acids dilutes linoleic acid, making it less effective in blocking enzyme synthesis. Our conclusions are therefore opposite to those of Jeffcoat *et al.* (1979), who proposed that the inhibition of fatty acid synthesis by linoleic acid occurred through an initial decrease in acyl-CoA desaturase activity, which in turn decreased fatty acid synthesis *de novo*. Lean mice made hyperphagic by treatment with gold-thioglucose have increased proportions of monounsaturated fatty acids (Soyka *et al.*, 1969), thus supporting the hypothesis that stimulation of the production of acyl-CoA desaturase by endogenous fatty acids may

be a general phenomenon and not restricted to the *ob/ob* genome.

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