The Role of Insulin in the Regulation of Stearic Acid Desaturase Activity in Liver and Adipose Tissue from Obese–Hyperglycaemic (*ob/ob*) and Lean Mice

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The relationship between the hyperinsulinaemia of obese–hyperglycaemic (*ob/ob*) mice and their high activity of stearic acid Δ^9 -desaturase compared with lean mice has been investigated. The concentrations of plasma insulin in obese mice were decreased by 71, 88 and 96% after treatment either with alloxan or food restriction to maintain the same weight as lean mice, or treatment of the weight restricted mice with alloxan followed by feeding *ad libitum*. The concentration of plasma insulin produced by the latter treatment was the same as in normal lean mice. After treatment the hepatic desaturase activities were 24, 68 and 19% less respectively on a cell basis than in livers from untreated obese mice, and the total epididymal fat-pad activities were lower by 16, 62 and 57%. These results suggest that hyperinsulinaemia is not essential for the increased hepatic desaturase, but may be important in adipose tissue. Food intake appears to be a significant factor controlling the hepatic desaturase activity, but even this may be subject to overriding regulation by the concentration of esterified linoleic acid in the liver lipids, which was negatively correlated ($r = 0.91$, $P < 0.001$) with desaturase activity.

The biosynthesis of oleic acid in animals occurs through the desaturation of stearoyl-CoA by a microsomal enzyme system (Marsh & James, 1962). The enzyme has been shown to be insulin-dependent, since its activity is low in diabetic animals and can be restored by insulin treatment (Gellhorn & Benjamin, 1964). Injection of insulin also increases the activity in livers of normal rats (Inkpen *et al.*, 1969). Increased synthesis of palmitoleic acid and oleic acid has been demonstrated in the liver and adipose tissue of obese–hyperglycaemic (*ob/ob*) mice (Winand *et al.*, 1968, 1969*a,b*, 1973) and it has been confirmed that this occurs through an increase in the activity of the desaturase enzyme system (Enser, 1975). Since obese mice are markedly hyperinsulinaemic (Stauffer *et al.*, 1967; Christophe *et al.*, 1959) this seemed a likely cause of the increased desaturase activity.

In addition to insulin, dietary fatty acids are recognized as regulating the activity of fatty acid Δ^9 -desaturase (Inkpen *et al.*, 1969; Mercuri *et al.*, 1974; Holloway & Holloway, 1975; Jeffcoat & James, 1978*a*). Polyunsaturated fatty acids decrease and saturated fatty acids increase the desaturase activity. Since the carcass lipids of obese mice contain lower proportions of polyunsaturated fatty acids than lean mice (Haessler & Crawford, 1965; Stein *et al.*, 1967; Winand *et al.*, 1969*b*; Bullfield, 1972), the increased desaturase activity might be explained by a decrease in their inhibitory activity. Food intake also regulates Δ^9 -desaturase activity (Inkpen *et al.*, 1969; Lee & Sprecher, 1971) with a decrease during starvation and an increase to

supranormal activities during refeeding. Although the effects of food intake could be ascribed to changes in plasma insulin, they might also act through the direct provision of saturated fatty acid substrates or the provision of substrates through synthesis *de novo*. Fatty acid synthesis and Δ^9 -desaturase activity are often closely correlated and frequently regulated in common (Wahle & Garton, 1972; Wahle, 1974; Jeffcoat & James, 1978*b*). In addition to a marked hyperphagia, obese mice also show high rates of fatty acid synthesis in both liver and adipose tissue (Bates *et al.*, 1955*a,b*; Mayer *et al.*, 1955; Shigeta & Shreeve, 1964; Shreeve *et al.*, 1967; Jansen *et al.*, 1967).

The present study was therefore designed to investigate the significance of insulin in the regulation of Δ^9 -desaturase activity, particularly in obese mice. Alloxan has been used to decrease the plasma insulin through destruction of the β -cells of the pancreas (Dunn & McLetchie, 1943; Solomon & Mayer, 1962; Mahler & Szabo, 1971; Nordenström *et al.*, 1973; Solomon *et al.*, 1974) and the effect on the desaturase activity of liver and epididymal fat-pad has been determined. To confirm that differences in activity represent differences in functional enzyme, the effects on the fatty acid composition of the tissues were also measured.

Materials and Methods

The animals and procedures used were as described by Enser (1972, 1975), except as follows. Only male

mice of the C57B1/6J line carrying the obese (*ob*) gene were used. For decreasing weight, obese mice at 7–8 weeks of age were placed on a restricted diet of 1–2.5 g of food/day fed in two portions at 08:30 h and 17:00 h. At 10–11 weeks their weights were similar to those of lean mice of the same age. The lean mice were homozygous normal mice at the (*ob*) locus, obtained from a subcolony of the obese line by selecting out the (*ob*) gene.

At 11–12 weeks of age all mice were weighed at 11:00 h and a sample of blood (20 μ l) was taken from the retrobulbar sinus for glucose determination. The animals were starved for 48 h and then injected with 0.01 unit of insulin. After 1 h the treated animals received alloxan (100 mg/kg body wt.; intraperitoneal injection in 0.1 ml of 0.9% NaCl). After treatment all animals were returned to feeding *ad lib*, except for the weight-decreased mice not treated with alloxan which remained on a restricted diet to maintain their weights at a constant value. At 4 days after treatment the mice were briefly anaesthetized with diethyl ether and another orbital blood sample was taken for glucose determination. At 11 days after treatment at 11:00 h the mice were anaesthetized with diethyl ether and as much blood as possible was obtained from the retrobulbar sinus and placed in a cold heparin-treated tube. The animals were killed by dislocation of the neck and the liver and epididymal fat-pads were rapidly removed. The tissues were processed by the method of Enser (1975) for the determination of stearic acid desaturase activity by measuring the conversion of [14 C]stearic acid into [14 C]oleic acid.

Food-intake measurements

Mice were kept singly and fed from hoppers designed to limit food spillage. The cages were cleaned out and residual food weighed every 3 to 4 days. Food intake is reported as the calculated average intake over the last 4 days before the animals were killed, since preliminary studies revealed that daily food intake was constant over this period. Desaturase activity was not determined on these mice, but they did not differ significantly from the experimental animals in body weight, liver weight or blood-glucose concentration.

Blood-glucose determination

The glucose oxidase/peroxidase method of Huggett & Nixon (1967) was used except that *o*-dianisidine, a potent carcinogen, was replaced by 2,2'-azinodi-(3-ethylbenzothiazoline-6-sulphonate).

Insulin determination

Insulin was determined on plasma prepared from heparin-treated blood with the radioimmunoassay

kit obtained from The Radiochemical Centre, Amersham, Bucks., U.K. With plasma from obese mice dilution studies revealed no differences between the binding of the mouse insulin and standard human insulin.

Chemicals

Analytical grade reagents were used if available and all solvents were purified by distillation. 2,2'-Azinodi-(3-ethylbenzothiazoline-6-sulphonate) was obtained from the Boehringer Corp., Lewes, Sussex, U.K. Alloxan monohydrate was obtained from BDH, Poole, Dorset, U.K.

Statistics

Analysis of variance and Student's *t* test were used to compare the significance of differences between results for different treatments. For wide-range results logarithmic transformations were used. *P* values of <0.05 were considered significant. Standard-linear-regression procedures were used.

Results

Food intake and body weight of mice

The food intake of all the alloxan-treated animals that survived stabilized after about 5 days and changed little subsequently. The calculated average daily food intake for all treatment groups measured over the period from 7 to 11 days after alloxan treatment is shown in Table 1. Alloxan-treated lean mice increased their food intake by 54% compared with untreated lean mice. Obese mice consumed 29% more food than lean mice, but alloxan treatment did not increase their food intake, although it was below that of alloxan-treated lean mice. Alloxan-treated obese mice after weight decrease ate more than untreated obese mice and alloxan-treated obese mice, but the difference was not significant.

At 12 weeks of age the growth of lean C57B1/6J mice is low, but that of obese mice is quite rapid with a gain of 1.7 g in 11 days (Table 1). Both the alloxan-treated lean and obese mice failed to recover the weight lost during the 48 h starvation before alloxan treatment and the lean mice continued to lose weight slowly. The alloxan-treated weight-decreased obese mice gained a little weight after treatment, but this was probably through an increase in the quantity of food in the intestine after they were returned to feeding *ad libitum*.

Effect of alloxan treatment on the plasma insulin, blood glucose and body weight

Alloxan-treated mice, whether obese, weight-decreased obese or lean, had plasma-insulin concentrations two-thirds less than untreated mice

of the same type (Table 1). In spite of this large decrease the plasma insulin of treated obese mice remained 8-fold higher than in lean mice. Although weight decrease and food restriction of obese mice was more effective than alloxan treatment alone in decreasing the plasma insulin the values obtained were still 3-fold higher than in lean mice. Only alloxan treatment after weight decrease was effective in returning the plasma insulin concentrations of obese mice to those found in untreated lean mice. No weight-decreased obese mice that survived the alloxan treatment had plasma-insulin concentrations lower than in untreated lean mice.

Alloxan-treated lean mice showed the expected increase in blood-glucose concentrations (Table 1). Obese mice were mildly hyperglycaemic with 22–32% more blood glucose than lean mice and alloxan produced an 8.5% decrease giving a concentration that was not significantly different from that in lean mice. Weight-restricted obese mice had a lower blood glucose than lean mice, corresponding to their decreased food intake, but after alloxan treatment and a return to feeding *ad libitum* they

became hyperglycaemic with twice the concentration of blood glucose found in obese mice, but less than in alloxan-treated lean mice. Some alloxan-treated weight-decreased obese mice had blood-glucose values similar to alloxan-treated lean mice 4 days after treatment, but these animals did not survive for 11 days.

Stearic acid desaturase activity of liver

The total stearic acid desaturase activity in livers from obese mice was 10-fold greater than in livers from lean mice (Table 2). Part of this difference was the result of the 3-fold greater liver weight, consisting of an increased fat-content (Table 3) and hyperplasia, with 60% more DNA (Table 2). On a cellular basis the activity was 6.5-fold higher in obese mice. Alloxan-treated obese mice had 31% less total liver activity, but because the DNA content of the liver was also less, the difference per cell was only 25%. Alloxan-treated lean mice had 86% less total liver activity than untreated lean mice, but the liver DNA content was the same.

Table 1. *Effect of alloxan treatment on body weight, plasma insulin, blood glucose and food intake of obese, weight-decreased obese and lean mice*

For experimental methods see the text. Results are means $\pm$ S.E.M. for ten mice in each group except for food intake with six mice per group. Numbers within columns with different superscript letters differ significantly at $P < 0.05$.

Type of mouse	Treatment	Body weight (g)		Blood glucose concn. (mm)		Final plasma insulin concn. (μ -units/ml)	Food intake (g/day)
		Initial	Final	Initial	Final		
Obese	Weight-decreased	27.1 $\pm$ 0.8 ^{a,b} NS†	26.7 $\pm$ 0.5 ^a	6.3 $\pm$ 0.2 ^a NS	6.6 $\pm$ 0.4 ^a	113 $\pm$ 23 ^a	2.3 $\pm$ 0.1 [‡]
Obese	Weight-decreased alloxan-treated	26.9 $\pm$ 0.5 ^{a,b} NS	28.7 $\pm$ 0.9 ^a	6.1 $\pm$ 0.2 ^{a****}	24.9 $\pm$ 1.9 ^d	35.4 $\pm$ 3.9 ^b	5.9 $\pm$ 0.4 ^{c,d}
Obese	None	47.9 $\pm$ 0.5 ^c NS	49.6 $\pm$ 0.8 ^b	12.7 $\pm$ 0.8 ^c NS	12.3 $\pm$ 0.6 ^c	952 $\pm$ 119 ^c	5.3 $\pm$ 0.3 ^c
Obese	Alloxan-treated	46.6 $\pm$ 0.4 ^{c*}	44.5 $\pm$ 0.8 ^c	11.7 $\pm$ 0.5 ^c NS	10.7 $\pm$ 0.8 ^{b,c}	278 $\pm$ 31 ^d	5.5 $\pm$ 0.2 ^c
Lean	None	26.0 $\pm$ 0.2 ^b NS	26.3 $\pm$ 0.3 ^d	9.6 $\pm$ 0.2 ^b NS	9.8 $\pm$ 0.6 ^b	34.4 $\pm$ 3.1 ^b	4.1 $\pm$ 0.1 ^a
Lean	Alloxan-treated	27.5 $\pm$ 0.6 ^{a****}	22.0 $\pm$ 0.6 ^d	10.3 $\pm$ 0.3 ^{d****}	40.9 $\pm$ 1.9 ^e	13.4 $\pm$ 2.7 ^e	6.3 $\pm$ 0.3 ^b

† Significance of difference between initial and final values: NS, not significant at $P > 0.05$; * $P < 0.05$; ***, $P < 0.001$. Initial results are before starvation, final results are 11 days after alloxan treatment.

‡ Weight of food fed to maintain this group of mice at constant weight.

Table 2. *Stearic acid desaturase activity, weight and DNA content of livers from alloxan-treated and control obese, weight-decreased obese and lean mice*

For experimental methods see the text. Results are means $\pm$ S.E.M. Numbers within columns with different superscript letters differ significantly at $P < 0.05$

Type of mouse	Treatment	Oleate formed (μ mol/liver per h)	Liver weight (g)	DNA (mg/liver)
Obese	Weight-decreased	8.98 $\pm$ 1.23 ^a	1.38 $\pm$ 0.08 ^a	4.37 $\pm$ 0.18 ^a
Obese	Weight-decreased alloxan-treated	22.88 $\pm$ 2.37 ^b	2.36 $\pm$ 0.09 ^b	4.38 $\pm$ 0.20 ^a
Obese	None	40.84 $\pm$ 2.24 ^c	4.17 $\pm$ 0.15 ^d	6.08 $\pm$ 0.43 ^b
Obese	Alloxan-treated	26.55 $\pm$ 2.01 ^b	2.81 $\pm$ 0.17 ^c	5.23 $\pm$ 0.27 ^c
Lean	None	3.94 $\pm$ 0.55 ^d	1.30 $\pm$ 0.03 ^a	3.80 $\pm$ 0.12 ^d
Lean	Alloxan-treated	0.54 $\pm$ 0.19 ^e	1.47 $\pm$ 0.04 ^c	3.82 $\pm$ 0.09 ^d

The desaturase activity of the weight-decreased obese mice was only 22% of that in obese mice, but because they had fewer liver cells the activity per cell was 31% of that in obese mice. Alloxan treatment did not change the liver DNA content, but after treatment the desaturase activity was 255% greater, reaching 60% of the activity of obese mice on a total tissue basis and 81% on a cellular basis. The activity in the weight-decreased alloxan-treated obese mice was not significantly different from that in the alloxan-treated obese mice on either a whole tissue or cellular basis.

Hepatic fat-content and fatty acid composition

The large differences in the activity of stearic acid desaturase between the groups of mice should affect the fatty acid composition of the liver lipids if the activity measured represents functional enzyme. The percentages of the substrate fatty acids, palmitic acid

and stearic acid (Paulsrud *et al.*, 1970), and the products palmitoleic acid and oleic acid in the total liver lipids are shown in Table 3. The high concentration of oleic acid in the liver lipids from obese mice was similar to that reported previously (Lemonnier *et al.*, 1971; Enser, 1975). Over all the treatment groups differences in desaturase activity corresponded to differences in the proportion of oleic acid and corresponded inversely with the proportion of stearic acid. The proportions of palmitic acid and palmitoleic acid did not show a clear pattern, but the proportion of palmitoleic acid in the liver lipids from alloxan-treated lean mice was 74% less than in untreated mice. Furthermore regression analysis revealed that when the percentages of palmitoleic acid and oleic acid were combined the variations in desaturase activity accounted for a higher percentage of the variance than for either fatty acid alone (Table 4 and Fig. 1).

Table 3. Fat content and fatty acid composition of liver lipids from alloxan-treated and untreated obese, weight-decreased obese and lean mice

For experimental methods see the text. Results are means $\pm$ S.E.M. for ten mice in each group. Numbers within columns with different superscript letters differ significantly at $P < 0.05$.

Type of mouse	Treatment	Fat-content (mg/liver)	Fatty acid composition (% by weight)					
			Palmitoleic acid	Oleic acid	Palmitic acid	Stearic acid	Linoleic acid	Arachidonic acid
Obese	Weight-decreased	56.6 $\pm$ 7.0 ^a	6.8 ^c	32.6 ^c	24.8 ^c	6.8 ^c	11.1 ^c	7.4 ^c
Obese	Weight-decreased alloxan-treated	248.2 $\pm$ 31.2 ^b	7.2 ^c	56.7 ^c	22.7 ^b	3.2 ^b	3.3 ^a	2.0 ^b
Obese	None	668.2 $\pm$ 43.8 ^c	8.7 ^d	58.8 ^c	21.7 ^{a,b}	2.1 ^a	3.0 ^a	1.0 ^a
Obese	Alloxan-treated	247.2 $\pm$ 42.1 ^b	9.4 ^c	48.7 ^d	25.2 ^c	3.3 ^b	5.1 ^b	2.5 ^b
Lean	None	59.4 $\pm$ 3.3 ^a	5.0 ^b	25.1 ^b	27.1 ^c	9.0 ^d	14.2 ^d	8.6 ^c
Lean	Alloxan-treated	53.0 $\pm$ 4.9 ^a	1.3 ^a	14.5 ^a	20.2 ^a	21.3 ^c	19.1 ^c	11.0 ^d

Table 4. Correlation coefficients and regression constants for the relationship between hepatic Δ^9 -desaturase activity and the proportions of hepatic fatty acids

x variable*	y variable	a	b	r	Variance accounted for (%)
Oleate formed (nmol/mg of protein per h)	Palmitoleic acid in total liver lipid (%)	2.572	0.061	0.858	73.2
Oleate formed (nmol/mg of protein per h)	Oleic acid in total liver lipid (%)	14.659	0.390	0.928	85.9
Oleate formed (nmol/mg of protein per h)	Palmitoleic acid + oleic acid in total liver lipid (%)	17.232	0.451	0.939	87.9
Linoleic acid in non-esterified fatty acids of liver (%)	Oleate formed (nmol/mg of protein per h)	140.691	-7.866	-0.864	74.1
Linoleic acid + arachidonic acid in non-esterified fatty acids of liver (%)	Oleate formed (nmol/mg of protein per h)	155.375	-4.281	-0.849	71.5
Linoleic acid in esterified fatty acids of liver (%)	Oleate formed (nmol/mg of protein per h)	117.346	-5.821	0.914	83.3
Linoleic acid + arachidonic acid in esterified fatty acids of liver (%)	Oleate formed (nmol/mg of protein per h)	115.749	-3.572	0.913	83.1

* Regression equation $y = a + bx$.

The proportion of linoleic acid and arachidonic acid, inhibitors of the desaturase enzyme, varied inversely with oleic acid in the total liver lipids. The liver lipids from obese mice contained only 3% linoleic acid and 1% arachidonic acid compared

with 19.1 and 11% respectively in lean mice. Weight-decreased obese mice had increased proportions of both fatty acids compared with untreated obese mice, but after alloxan treatment and refeeding linoleic acid was 3.3% and arachidonic acid was 2% of the liver fatty acids, similar to the values for untreated obese mice.

The fat-content of the livers was closely correlated with the liver weight ($r = 0.876$, $P < 0.001$) (Table 3). Weight-decreased obese mice had similar liver fat-contents to the lean and alloxan-treated lean mice. Alloxan-treated obese mice had 63% less liver fat than untreated obese mice, but similar quantities to the alloxan-treated weight-decreased mice. This was 4 times more than in untreated weight-decreased obese mice or lean mice.

Stearic acid desaturase activity of epididymal fat-pads

The stearic acid desaturase activity of the epididymal fat-pads of untreated obese mice was 4.6 times higher than in lean mice (Table 5). Weight-decreased obese mice also had a higher activity than lean mice (1.7-fold), but it was not changed after alloxan treatment, although the weight of the fat-pad was 20% less. Alloxan-treated obese mice had less desaturase than untreated mice, but the difference was not significant, although the fat-pads were significantly smaller (14%). Alloxan-treated lean mice had no visible fat-pad and the activity could not be determined.

The fatty acid composition of the epididymal fat-pad lipids revealed characteristically low proportions of linoleic acid in all the groups of obese mice compared with lean mice, and alloxan treatment had no effect. Although the fat-pads of weight-decreased obese mice were only 45% of the weight of those from obese mice, the linoleic acid content was only 13% greater. In spite of the increased desaturase activity, all the obese mouse groups had similar proportions of stearic acid, which was 74–88% greater than in lean mice. Palmitoleic acid was

Fig. 1. Relationship between mouse hepatic stearic acid desaturase activity and the combined proportions of palmitoleic acid and oleic acid in the total liver lipids

For the regression constants see Table 4. For experimental details see the text. Symbols: Δ , weight-decreased obese mice; $\blacktriangle$, weight-decreased alloxan-treated obese mice; $\square$, obese mice; $\blacksquare$, alloxan-treated obese mice; $\circ$, lean mice; $\bullet$, alloxan-treated lean mice.

Table 5. Effect of alloxan treatment on stearic acid desaturase, fatty acid composition and weight of epididymal fat-pads from obese, weight-decreased obese and lean mice

For experimental details see the text. Results are means $\pm$ S.E.M. for ten mice in each group. Numbers within a column with different superscripts letters differ significantly at $P < 0.05$.

Type of mouse	Treatment	Oleic acid formed (n mol/two fat-pads per h)	Fat-pad weight (g)	Fatty acid composition (% by weight)				
				Palmitoleic acid	Oleic acid	Palmitic acid	Stearic acid	Linoleic acid
Obese	Weight-decreased	57.2 $\pm$ 3.7 ^a	1.48 $\pm$ 0.06 ^a	12.1 ^b	45.3 ^{a,b}	23.7 ^a	2.89 ^a	12.4 ^a
Obese	Weight-decreased alloxan-treated	63.9 $\pm$ 9.8 ^a	1.19 $\pm$ 0.07 ^b	11.5 ^b	46.8 ^a	23.6 ^a	2.78 ^a	12.4 ^a
Obese	None	151.2 $\pm$ 18.7 ^b	3.28 $\pm$ 0.11 ^c	13.3 ^{a,b}	43.0 ^c	25.8 ^b	2.73 ^a	11.0 ^b
Obese	Alloxan-treated	127.5 $\pm$ 10.3 ^b	2.82 $\pm$ 0.10 ^d	14.1 ^a	44.1 ^b	23.5 ^a	2.95 ^a	11.3 ^{a,b}
Lean	None	39.2 $\pm$ 6.6 ^c	0.42 $\pm$ 0.02 ^e	10.1 ^c	41.0 ^c	22.6 ^a	1.57 ^b	19.9 ^c

significantly higher in all the obese groups than the lean groups and alloxan treatment again had no effect. Oleic acid was slightly higher in all the groups of obese mice, but not significantly so in the untreated obese mice. Alloxan treatment resulted in higher proportions of oleic acid, but the difference was not significant in the weight-decreased alloxan-treated obese mice.

Discussion

The present study clearly shows that hyperinsulinaemia is not a prerequisite for the high activity of stearic acid desaturase observed in livers from obese-hyperglycaemic mice, since obese mice whose body weight and plasma insulin had been decreased to that of lean mice by food restriction and alloxan treatment had 5 times more desaturase activity in their livers than lean mice. Furthermore, over half of this activity appeared after the plasma insulin had been returned to normal concentrations. There was also a lack of correlation between plasma-insulin concentrations and liver desaturase activity in obese mice, since a 60% difference in plasma insulin caused by alloxan treatment was associated with only a 23% difference in desaturase activity. The decrease (88%) in the desaturase activity of livers from alloxan-treated lean mice was similar to that reported in rats (Gellhorn & Benjamin, 1964), but I was unable to test whether the desaturase activity of livers from obese mice was insulin-dependent at insulin concentrations less than in lean untreated mice, since no obese mice with such low plasma insulin survived the treatment.

The stimulus for the increase in the hepatic desaturase activity of the alloxan-treated weight-decreased mice is not known. In rats, refeeding after starvation or feeding saturated fatty acids increases the Δ^9 -desaturase activity without sustained increases in plasma insulin (Inkpen *et al.*, 1969). Furthermore, even in diabetic rats the desaturase activity can be increased by feeding fructose, glycerol or saturated fatty acids (Mercuri *et al.*, 1974). These results suggest that provision of substrate is able to induce desaturase activity. The major source of substrate in the mouse is through fatty acid synthesis *de novo*, since the diet is low in fat and contains mainly unsaturated fatty acids. It has also been suggested that fatty acid synthetase and Δ^9 -desaturase are frequently regulated in concert (Wahle & Garton, 1972; Wahle, 1974; Jeffcoat & James, 1978b). I did not determine fatty acid synthesis, but it has been reported that obese mice treated with streptozotocin or anti-insulin serum, so that their plasma insulin is only twice as high as in lean mice, have rates of hepatic fatty acid synthesis similar to lean mice (Assimakopoulos-Jeannet *et al.*, 1974; Batchelor *et al.*, 1975). In the alloxan-treated obese mice it is

possible that the 23% decrease in hepatic desaturase activity was caused by a decrease in fatty acid synthesis, especially as the blood glucose of these mice was decreased toward that of lean mice and their liver fat content was less than in untreated mice. However, their food intake was not decreased. The low desaturase activity of the weight-decreased obese mice might also have been caused by a decrease in fatty acid synthesis (Hems & Ma, 1976), but on return to feeding *ad libitum* after alloxan treatment their food intake returned to the value of untreated obese mice. Fatty acid synthesis might have returned to high values in spite of the diabetic condition of these mice, since this enzyme system has been shown to be resistant to regulation by vasopressin or starvation in obese mice (Hems & Ma, 1976; Volpe & Marasa, 1975).

In the epididymal fat-pad the effect of insulin on the desaturase activity could be indirect through the provision of substrates for fatty acid synthesis. The adipose tissue of obese mice, unlike muscle, is believed to be sensitive to insulin and in a constantly stimulated state due to the hyperinsulinaemia (Chlouverakis & White, 1969; Clarke *et al.*, 1975; Loten *et al.*, 1976). It is therefore not surprising that there was little difference between the desaturase activity of fat-pads from obese and alloxan-treated obese mice. Although the latter had 60% lower plasma-insulin concentrations, these were 8-fold higher than in lean mice. However, the weight of the fat-pads was 14% lower in the alloxan-treated mice and their body weights were not increasing, although they ate as much as the untreated mice, which might suggest that adipose tissue lipogenesis was decreased.

If the sensitivity of adipose tissue from obese mice to insulin is normal it may explain why the desaturase activity in epididymal fat-pads from weight-decreased alloxan-treated mice was similar to that in untreated mice when the liver activity was much higher. If allowance is made for the 40–50% increase in the number of fat-cells in the epididymal fat-pads in obese mice (Johnson & Hirsch, 1972) the activity per cell in alloxan-treated weight-decreased obese mice was not significantly different from that in tissue from lean mice in the presence of similar concentrations of plasma insulin. However, the hyperglycaemia of these mice and their failure to gain weight after alloxan treatment, although consuming similar quantities of food to alloxan-treated lean mice, argues against normal adipose tissue fatty acid synthesis and metabolism.

Fatty acid Δ^9 -desaturase activity is decreased by polyunsaturated fatty acids *in vivo* and *in vitro* (Uchiyama *et al.*, 1967; Inkpen *et al.*, 1969; Bickerstaffe & Annison 1970; Jeffcoat & James, 1978a), and since obese mice have lower proportions of polyunsaturated fatty acids in their carcass lipids

than lean mice this might explain some of the differences in desaturase activity. Previously I failed to find any relationship between the linoleic acid content of the non-esterified fatty acid pool of the liver and desaturase activity (Enser, 1975). In the present study the proportion of linoleic acid in the non-esterified fatty acids of the liver accounted for 74% of the variance of the desaturase, but the proportion of esterified linoleic acid accounted for 83% of the variance taken over all the groups of mice (Table 4 and Fig. 2). No other fatty acid either polyunsaturated or a substrate of the enzyme whether alone or in combination was a better predictor of desaturase activity. The likely regulator is either a linoleic acid-containing lipid or non-esterified linoleic acid in a specific pool. Wahle & Radcliffe (1977) observed similar desaturase activities in the livers from lean and fatty rats when the proportions of linoleic acid in the microsomal fraction were equalized by feeding sunflower oil to the obese rats.

The differences in the fatty acid composition of the fat-pads between all groups of obese mice were small and there was no clear relationship between fatty acid composition and desaturase activity. This

was not unexpected, because of the slow turnover of the stored triacylglycerol fatty acids and the net loss of lipid from the fat-pads of the alloxan-treated groups. Neither was there any good relationship between the proportion of non-esterified linoleic acid in the fat-pad and the desaturase activity, since the proportions were not significantly different from the weight-decreased mice, the alloxan-treated weight-decreased mice and the untreated obese mice (results not shown). Although these results do not support a role for linoleic acid in the regulation of adipose tissue desaturase activity such a role cannot be ruled out, since I have observed a decrease in adipose tissue desaturase of obese mice fed corn oil similar to the reported changes in rat liver desaturase (Inkpen *et al.*, 1969).

Although the present study demonstrates that hepatic desaturase activity can be increased in the absence of hyperinsulinaemia, the role of insulin in the regulation of adipose tissue desaturase is less clear. Further studies are needed to determine the relationship between insulin, fatty acid synthesis and desaturase activity in adipose tissue.

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Fig. 2. Relationship between the proportion of linoleic acid present in mouse hepatic lipids and the activity of stearic acid desaturase

For the regression constants see Table 4. For experimental details see the text. Symbols: Δ , weight-decreased obese mice; $\blacktriangle$, weight-decreased alloxan-treated obese mice; $\square$, obese mice; $\blacksquare$, alloxan-treated obese mice; $\circ$, lean mice; $\bullet$, alloxan-treated lean mice.

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