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Desaturation of Stearic Acid by Liver and Adipose Tissue from Obese–Hyperglycaemic Mice (*ob/ob*)

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1. Stearic acid desaturase activity was assayed in preparations from perigenital adipose tissue and liver from lean and genetically obese female mice (*ob/ob*). 2. The total activity in the perigenital adipose tissue from obese mice was threefold greater than in the tissue from lean mice, but per g of adipose tissue the activity was twofold greater in tissue from lean mice. 3. In liver, the activity in obese mice was elevated at 8 weeks of age, remained elevated up to 24 weeks and then decreased by half at 48 weeks, but at all ages was higher than that in lean mice. 4. The decrease in desaturase activity of liver from obese mice at 48 weeks corresponded to a change in the fatty acid composition of liver lipids toward that found in lean mice. 5. Whereas in adipose tissue much of the increased enzyme activity may be due to tissue hyperplasia, in liver it is mainly an increased activity per cell.

Mice which are homozygous for the obese gene (*ob*) are characterized by excessive deposition of body fat amounting to about a fivefold higher body fat content than their lean counterparts. The only consistent change in fatty acid composition, however, is an increase in the proportion of palmitoleic acid (Haessler & Crawford, 1965; Stein *et al.*, 1967; Winand *et al.*, 1969*b*; Bullfield, 1972) with a compensatory small decrease in several fatty acids but mainly linoleic acid. The obese strains show both a high capacity to deposit dietary fat (Lemonnier *et al.*, 1971) and a higher lipogenic capacity (for a review see Bray & York, 1971), which may maintain the fatty acid composition near normal since mouse diets usually contain a lower proportion of oleic acid than linoleic acid, the reverse of the proportions in adipose tissue. Either oleic acid and palmitoleic acid must be stored preferentially or their synthesis increased.

The final step in the synthesis of oleic acid is the desaturation of stearoyl-CoA by a microsomal enzyme system, in liver or adipose tissue, which also desaturates palmitoyl-CoA (Marsh & James, 1962; Gellhorn & Benjamin, 1964). The enzyme is insulin-dependent since its activity is lower in diabetic animals and can be restored by insulin treatment (Gellhorn & Benjamin, 1964); it can also be increased in normal fed animals by injection of insulin (Inkpen *et al.*, 1969). Obese mice of the *ob/ob* strain are hyperinsulinaemic (Stauffacher *et al.*, 1967), and this together with the increase in palmitoleic acid indicates that they might have an enhanced fatty acid desaturase activity. Indeed Christophe and his colleagues (Winand *et al.*, 1968, 1969*a*,*b*, 1973) have observed an increased conversion of glucose, acetic acid and palmitic acid into palmitoleic acid and oleic acid both *in vivo* and *in vitro* in whole-tissue preparations from obese mice compared with their lean controls. However, since the desaturase enzyme was not assayed directly it was possible that a variation in other processes could have produced such results.

In the present study the conversion of stearic acid into oleic acid has been measured under optimal conditions *in vitro* by using enzyme preparations from both the liver and adipose tissue of obese and lean mice, since the liver of obese mice is of more significance in lipogenesis than in lean mice (Jansen *et al.*, 1967).

Materials and Methods

Animals

Mice of the C57Bl/6J strain carrying the obese (*ob*) gene were bred in our own colony as described previously (Enser, 1972). Female mice kept under controlled illumination, 12h light, 12h dark, were used for all studies.

Tissue preparations

The liver was homogenized with 0.25M-sucrose containing 50mM-potassium phosphate buffer, pH 7.4 (4ml/g), at 4°C by using a Teflon–glass homogenizer. After centrifuging at 1500*g* for 15min at 4°C the supernatant was removed and filtered through glass wool to remove fat-particles. It was then centrifuged at 15000*g* for 15min. Fat-pads were homogenized under the same conditions except that a minimum of 3ml of the sucrose–phosphate buffer was used. After being left on ice for 5min the homogenate was

centrifuged at 1500*g* at 4°C. The infranatant was removed and filtered through glass wool.

Stearic acid desaturase assay

An assay based on the method of Gellhorn & Benjamin (1964) was used to determine the conversion of stearic acid into oleic acid. Samples of the liver enzyme preparation, diluted 1 to 30 with sucrose–phosphate buffer, were added to a tube, cooled on ice, containing (final concentrations) 20μM-[1-^{14}C]-stearic acid (4mCi/mmol; dissolved in 20μl of propylene glycol), 10mM-ATP, 0.1mM-CoA, 6mM-NADH, 0.2mM-NADPH, 20mM-DL-α-glycerophosphate, 10mM-$MgCl_2$, 40mM-NaF and 50mM-potassium phosphate buffer, pH7.4. The volume was made to 1.0ml with 0.25M-sucrose containing 50mM-potassium phosphate buffer, pH7.4. The tube was gassed with O_2 for 30s and incubated in a shaking water bath at 35°C for 10min.

The adipose-tissue preparation was incubated in a tube containing 100μM-[1-^{14}C]stearic acid (8mCi/mmol), 10mM-ATP, 0.15mM-CoA, 5mM-$MgCl_2$, 10mM-DL-α-glycerophosphate and 50mM-potassium phosphate buffer, pH7.4, in a volume made to 0.9ml with 0.25M-sucrose containing 50mM-potassium phosphate buffer, pH7.4. After being gassed with O_2 for 30s the tubes were capped and incubated in a shaking water bath at 35°C for 5min. A solution of NADH and NADPH (0.1ml), to give final concentrations of 6mM and 0.2mM respectively, was then injected through the caps and the incubation continued for 10min. Time-control assays were terminated before the addition of NADH and NADPH. The assays of the liver enzyme were stopped by the addition of 0.3ml of 10M-NaOH and 1ml of methanol containing 0.1% (w/v) quinol. After the addition of 0.1ml of diethyl ether containing oleic acid (0.5mg), palmitic acid (0.25mg) and stearic acid (0.25mg) the tubes were heated at 60°C for 2h. The mixture was acidified with 0.4ml of 5M-H_2SO_4, and 3ml of water was added. The fatty acids were extracted three times with 3ml of petroleum spirit (b.p. range 40–60°C), and, after reduction of the volume, methyl esters were prepared by the addition of an ethereal solution of diazomethane (Getz & Bartley, 1961). The assay of adipose-tissue enzyme was treated similarly except that only 0.2ml of 10M-NaOH was added, the saponification was for 1h and 0.3ml of 5M-H_2SO_4 was used for acidification.

The saturated and monounsaturated fatty acids were separated on thin layers of silica gel G (Polygram sheet no. PG22, Macherey-Nagel and Co., obtained from Camlab Ltd., Cambridge, U.K.) impregnated with $AgNO_3$. The plates were developed in 1,1,2,2-tetrachloroethane–propan-2-ol (20:1, v/v) in unlined tanks, and the spots were made visible by spraying with 2,7-dichlorofluorescein. The spots were cut out and placed in vials for scintillation counting. Water (1ml) was added to deactivate the silicic acid followed by 10ml of scintillation fluid (Kaplan, 1970). Samples were counted for radioactivity to 2000 total counts at an efficiency of 72%. The recovery of counts from the t.l.c. plates was 95–105%. Each tissue was assayed in duplicate together with appropriate controls, and samples from lean and obese mice of the same age were assayed together. The assay of liver desaturase was linear for 15min and the enzyme activity was proportional to protein concentration between 1 and 5mg/ml. The assay of adipose-tissue desaturase was linear for 20min after the addition of NADH and NADPH at protein concentrations from 0.15–0.9mg/ml. If, however, the initial incubation was omitted there was a 2–3min lag in the synthesis of oleic acid.

Determination of the fatty acid composition of tissue lipids

Lipids were extracted from a sample of the complete liver homogenate by the method of Folch *et al.* (1957). Portions of the lipid were saponified in 2M-KOH in aq. 50% (v/v) methanol at 60°C for 1h. After acidification the fatty acids were extracted into light petroleum (b.p. range 40–60°C), methylated with diazomethane in ether, and the composition was determined by g.l.c.

Non-esterified stearic acid content of the enzyme preparations

Lipid was extracted from the enzyme preparations in Dole's extraction mixture (Dole & Meinertz, 1960) containing a standard amount of pentadecanoic acid. The extract was evaporated to dryness and the lipid redissolved in heptane–chloroform (4:3, v/v). The non-esterified fatty acids were then extracted with three washes of 0.5% (w/v) K_2CO_3 in aq. methanol (1:1, v/v). After acidification the fatty acids were extracted back into petroleum spirit, methylated and analysed by g.l.c. The quantity of stearic acid present was determined by comparison of its peak area with that of the internal standard pentadecanoic acid. The linoleic acid content of the enzyme preparations was determined in the same way.

Gas–liquid chromatography

Fatty acid methyl esters were separated on columns [1.52m×0.64cm diam. (5ft×0.25in diam.)] packed with 10% polyethylene glycol adipate on Gas-Chrom Q on a Pye 104 chromatograph with a flame-ionization detector operated isothermally at 186°C. The fatty acids were identified by comparison of the

retention time with known standards and the composition was determined by triangulation of the peak areas.

Protein determination

Protein was determined by the method of Lowry *et al.* (1951) with crystalline bovine albumin as a standard.

DNA determination

DNA was determined by the diphenylamine method of Burton (1956), with calf thymus DNA as standard, after extraction of the lipid with ethanol and diethyl ether.

Chemicals

If possible, analytical-grade reagents were used, and all solvents were purified by distillation. [1-^{14}C]Stearic acid was obtained from The Radiochemical Centre, Amersham, Bucks., U.K. and was used without further purification. ATP, NADH, NADPH and CoA were from Boehringer Corp. (London) Ltd., London W.5, U.K. Polyethyleneglycol adipate was from Pye–Unicam Ltd., Cambridge, U.K. and Gas Chrom Q (Applied Science Laboratories) was from Field Instruments Co., Richmond, Surrey, U.K.

Results

Stearic acid desaturase of perigenital fat-pad

When expressed on the basis of tissue protein there was no difference in the desaturase activity of perigenital fat-pads from 12-week-old female lean and obese mice (Table 1); on the basis of fresh tissue weight the activity in the lean mice was 2.4 times greater than that in the obese mice. However, the fat-pads from obese mice were seven times larger than those from lean mice, so that the total fat-pad activity in the obese mice was 3.3 times higher.

No significant difference in the non-esterified stearic acid content of the enzyme preparations from lean and obese mice was observed (Table 1), and dilution of the substrate was not taken into account in calculating the results: if the stearic acid present in the homogenate is allowed for, the absolute activities were 5.5–6.5% higher.

Table 1. *Stearic acid desaturase activity of the perigenital fat-pad from lean and obese mice*

For experimental details see the text. Results are means ±S.E.M. for nine 12-week-old female mice in each group. *$P<0.05$, **$P<0.001$.

Type of mouse ...	Lean	Obese
Oleate formed (nmol/h per mg of protein)	25.5±4.4	23.4±1.9
Oleate formed (nmol/h per g of tissue)	296.6±65.3	126.5±12.4*
Oleate formed (nmol/h per perigenital fat-depot)	76.9±17.9	245.2±19.3**
Weight of mice (g)	21.6±0.4	41.8±0.8**
Weight of perigenital fat-depot (g)	0.27±0.03	1.97±0.07**
Non-esterified stearic acid (nmol/ml of enzyme)	14.3±1.7	16.6±3.7

Stearic acid desaturase activity of liver

The stearic acid desaturase was more active in the livers from obese mice from 8 to 48 weeks of age, than in livers from lean mice (Table 2). The activity per mg of protein did not change between 8 and 24 weeks of age in obese mice but by 48 weeks it had decreased by half. This decrease was accompanied by an increase in the DNA per g of wet tissue and a decrease in the fat content (Table 2). In livers from lean mice the stearic acid desaturase activity per mg of protein was similar at 8, 12 and 48 weeks of age but had doubled at 24 weeks of age. At this age the greatest variation in activity between animals was observed and it corresponds to a slight decrease in the fat content of the liver. The weight of the livers from lean mice increased by 22% between 8 and 24 weeks and remained the same up to 48 weeks of age. The livers from obese mice were 2.6 times heavier than those from lean mice at 8 weeks of age, and between 8 and 24 weeks they increased by 74%. The weight then decreased and at 48 weeks it was only 21% greater than at 8 weeks. The difference in liver weights between lean and obese mice resulted in a desaturase activity 5.4–9.4-fold higher in the obese mice (Table 2). The difference in desaturase activity was not caused by differential dilution of the substrate since the quantity of non-esterified stearic acid present in the enzyme preparations was similar in lean and obese mice and showed no consistent variation with age. Since linoleic acid has been reported to inhibit the desaturase, and because of the presence of a decreased proportion of linoleic acid in the tissues of obese mice the concentration of linoleic acid, in the enzyme preparations, was also determined. The quantity of non-esterified linoleic acid was indeed lower in enzyme preparations from 8- and 12-week-old obese mice compared with enzyme from lean mice of the same age (Table 2). However, at 24 weeks of age the linoleic acid content of the enzyme preparation from obese mice had increased and there was some overlap between the concentration in lean and obese mice. At 48 weeks of

Table 2. *Stearic acid desaturase activity of livers from lean and obese mice*

For experimental details see the text. Results are means±S.E.M. for nine female mice in each group. Unless otherwise indicated differences between lean and obese mice, at a given age, were significant, $P<0.001$ by Student's t test. *$P<0.01$; **not significant, $P>0.05$.

	Type of mouse	Age of mice (weeks) ... 8	12	24	48
Oleic acid formed (nmol/h per mg of protein)	Obese	96.3±4.4	100.3±4.1	104.0±3.8	47.9±6.4*
	Lean	25.4±2.0	29.5±6.0	64.1±8.6	23.8±2.0
Oleic acid formed (μmol/h per g of tissue)	Obese	9.9±0.4	10.3±0.5	9.7±0.4*	5.4±0.6
	Lean	2.7±0.2	2.7±0.4	6.5±1.0	2.7±0.2
Oleic acid formed (μmol/h per liver)	Obese	23.37±2.02	26.28±1.72	39.92±3.55	15.70±2.64
	Lean	2.47±0.19	2.80±0.42	7.43±1.19	2.94±0.20
Weight of mice (g)	Obese	31.4±1.0	40.2±0.9	57.9±0.7	70.7±1.0
	Lean	18.7±0.3	23.4±1.0	24.2±1.8	27.5±0.5
Weight of liver (g)	Obese	2.35±0.15	2.58±0.18	4.09±0.24	2.84±0.19
	Lean	0.92±0.02	1.03±0.04	1.12±0.08	1.11±0.03
DNA concentration in liver (mg/g of wet tissue)	Obese	1.74±0.21	1.73±0.08	1.77±0.09	2.89±0.28**
	Lean	3.47±0.11	3.38±0.17	3.05±0.25	3.69±0.28
Fat concentration in liver (mg/g of wet tissue)	Obese	90.9±6.8	120.5±11.1	120.4±10.2	78.0±7.2*
	Lean	46.4±2.5	40.4±4.4	36.0±3.4	48.6±4.0
Stearic acid (nmol/ml of enzyme preparation)	Obese	2.01±0.17**	2.45±0.27**	1.93±0.26**	2.60±0.51**
	Lean	1.96±0.11	2.29±0.24	2.29±0.24	2.52±0.32
Linoleic acid (nmol/ml of enzyme preparation)	Obese	17.93±1.85	21.80±2.84	26.24±5.69**	41.80±8.02**
	Lean	36.66±2.52	44.22±2.29	36.50±4.82	42.30±8.02

age the concentration of non-esterified linoleic acid had increased further in the enzyme preparation from obese mice and was the same as that from lean mice.

Fatty acid composition of liver from lean and obese mice

The livers from obese mice contained between 168 and 452 mg more fat than the livers from lean mice, with the greatest and least differences at 24 and 48 weeks of age respectively (Table 2). To determine whether the decreased desaturase activity in obese mice at 48 weeks affected the liver fatty acids the fatty acid composition was determined. In the livers from lean mice there was no change in the fatty acid composition from 8 to 48 weeks, whereas in obese mice the fatty acid composition was the same at 8, 12 and 24 weeks, but had changed significantly at 48 weeks. The percentage composition of the major fatty acids in the livers from 24-week-old lean mice were as follows with the values for 24 and 48-week-old obese mice respectively in parentheses; palmitic acid 24.0 (25.0, 22.9); palmitoleic acid 4.6 (8.0, 8.1); stearic acid 8.1 (2.5, 5.0); oleic acid 26.4 (53.2, 43.4); linoleic acid 15.2 (5.4, 9.4); eicosenoic acid 0.6 (1.4, 0.8); arachidonic acid 8.1 (1.2, 3.3); docosahexaenoic acid 7.6 (1.3, 4.0). Except for palmitic acid the differences between fatty acids from the lean and obese mice were significant ($P<0.001$) as were the differences between 24- and 48-week-old obese mice except for palmitic acid and palmitoleic acid which did not change. Except for these two fatty acids the fatty acid composition of the lipids from the 48-week-old obese mice had changed toward that in the lean mice.

Discussion

The results of the direct assay of stearic acid desaturase reported in this paper demonstrate that the increased incorporation of precursors into palmitoleic acid and oleic acid observed by Christophe's group in whole-tissue preparations from obese mice are due, at least in part, to an increase in the fatty acid desaturase system (Winand *et al.*, 1968, 1969*a*, 1973). The threefold increase in the activity of stearic acid desaturase on a protein basis is similar to the increase in citrate cleavage enzyme (Kornacker & Lowenstein, 1964) acetyl-CoA carboxylase and fatty acid synthetase (Chang *et al.*, 1967) in livers from 12-week-old obese–hyperglycaemic mice compared with lean mice. Enzyme activities have not been reported for perigenital adipose tissue, but the conversion of acetate into fatty acids by epididymal fat-pads *in vitro* is increased fivefold on a tissue nitrogen basis (Hollifield *et al.*, 1960; Christophe *et al.*, 1961). However, *in vivo* Jansen *et al.* (1967) observed that the conversion of glucose into fatty acids per unit weight of tissue was 50% less in obese mice, similar to the results reported here for the desaturase.

In 8- and 12-week-old mice the liver DNA content is one-third higher than in the lean mice, and at 24 and

48 weeks it is twice as high. If the kinetic parameters of the enzyme are unchanged, this hyperplasia of the liver could only account for 4–23% of the increased desaturase activity. Differences in the quantity of non-esterified linoleic acid present in the assay might be responsible for some of the increased activity since desaturase activity has been shown to vary inversely with the quantity and degree of unsaturation of the fatty acids present in the assay (Uchiyama *et al.*, 1967; Bickerstaffe & Annison, 1970). However, the enzyme preparation from livers from 48-week-old obese mice had the same concentration of non-esterified linoleic acid as that from the livers from the lean mice, but twice as high a desaturase activity. The most likely explanation for the increased desaturase activity is the high plasma insulin concentrations found in obese mice (Stauffacher *et al.*, 1967). The decreased desaturase activity observed in the 48-week-old obese mice fits this hypothesis since the plasma insulin concentration is lower in obese mice of this age (Herberg *et al.*, 1970).

The perigenital fat-pads of 24-week-old female obese mice contain twice as many cells with an average content of lipid six times larger than the fat-pads from lean mice (Johnson & Hirsch, 1972). If it is assumed that the number of cells, although not their size, is the same at 12 and 24 weeks, hyperplasia could be responsible for half of the increased desaturase activity.

The fatty acid composition of livers from 48-week-old obese mice compared with 24-week-old mice showed the changes which might be expected if the fatty acid desaturase activity were a significant factor in determining the fatty acid composition of the tissue. However, the failure of the liver fatty acids to change after the increase in the desaturase activity in 24-week-old lean mice indicates the importance of other factors.

Although the results presented here establish a significant increase in the stearic acid desaturase activity in the liver and adipose tissue of obese mice it remains to be shown whether this is due to an increase in the quantity of enzyme or a change in its kinetic parameters.

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