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Fatty Acid Synthesis in Liver and Adipose Tissue of Normal and Genetically Obese (*ob*/*ob*) Mice during the 24-Hour Cycle

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1. The synthesis of long-chain fatty acids *de novo* was measured in the liver and in regions of adipose tissue in intact normal and genetically obese mice throughout the daily 24h cycle. 2. The total rate of synthesis, as measured by the rate of incorporation of ^3H from ^3H_2O into fatty acid, was highest during the dark period, in liver and adipose tissue of lean or obese mice. 3. The rate of incorporation of ^{14}C from [U-^{14}C]glucose into fatty acid was also followed (in the same mice). The $^{14}C/^3H$ ratios were higher by a factor of 5–20 in parametrial and scapular fat than that in liver. This difference was less marked during the dark period (of maximum fatty acid synthesis). 4. In normal mice, the total rate of fatty acid synthesis in the liver was about twofold greater than that in all adipose tissue regions combined. 5. In obese mice, the rate of fatty acid synthesis was more rapid than in lean mice, in both liver and adipose tissue. Most of the extra lipogenesis occurred in adipose tissue. The extra hepatic fatty acids synthesized in obese mice were located in triglyceride rather than phospholipid. 6. In adipose tissue of normal mice, the rate of fatty acid synthesis was most rapid in the intra-abdominal areas and in brown fat. In obese mice, all regions exhibited rapid rates of fatty acid synthesis. 7. These results shed light on the relative significance of liver and adipose tissue (i.e. the adipose 'organ') in fatty acid synthesis in mice, on the minor importance of glucose in hepatic lipogenesis, and on the alterations in the rate of fatty acid synthesis in genetically obese mice.

Rates of synthesis of fatty acids in liver and adipose tissue throughout the daily 24h cycle have not been satisfactorily documented. This requires a technique that measures the total rates of synthesis independently of the contributions of the manifold carbon sources. Such a method involves measuring the rate of incorporation of ^3H from ^3H_2O into lipids (see, e.g., Windmueller & Spaeth, 1967; Jungas, 1968; Salmon *et al.*, 1974).

From many previous experiments, mainly with ^{14}C-labelled precursors, it has appeared that fatty acid synthesis in the liver of rats or mice is a relatively minor process, compared with that in adipose tissue. However, measurements with ^3H_2O have shown that in rodents ingesting carbohydrate-based diets, the hepatic synthesis *de novo* of long-chain fatty acids may be a rapid process, of the same order as the tricarboxylic acid cycle (Lowenstein, 1971; Brunengraber *et al.*, 1973; Salmon *et al.*, 1974; see also McGarry & Foster, 1972). Hence a re-appraisal of the relative importance of fatty acid synthesis in the liver and adipose tissue of animals maintained on standard diets may be indicated.

The results reported in the present paper show that fatty acid synthesis, as measured with ^3H_2O and calculated per whole mouse, is more extensive in liver than in free adipose tissue (i.e. those macroscopically discrete areas of fat that lie apart from other organs), and expand previous work in that measurements were made throughout the daily 24h cycle. Also, experiments with [^{14}C]glucose are described which show that glucose makes a minor carbon contribution to hepatic fatty acid synthesis, especially during the daylight hours. Finally, measurements of the total rates of fatty acid synthesis in genetically obese (*ob*/*ob*) mice are presented. In these animals, there is enhanced lipogenesis in liver, as has been shown with ^{14}C-labelled precursors *in vivo* (Jansen *et al.*, 1967; Shreeve *et al.*, 1967; Salmon & Hems, 1973; Winand *et al.*, 1973; Elliott *et al.*, 1974; Loten *et al.*, 1974) and with ^3H_2O in the perfused liver (Assimacopoulos-Jeannet *et al.*, 1974). Nevertheless, the present results suggest that, at all ages, and at all times of the day, adipose tissue is the main site of the excess fatty acid synthesis in obese mice which are ingesting a starch-based diet.

Materials and Methods

Animals

Female mice were obtained from a random-bred closed colony into which the *ob* gene was incorporated (Abraham & Beloff-Chain, 1971). They were

fed on a standard mixed supplemented carbohydrate-based (Thompson's) breeding diet (Oxoid Ltd., London S.E.1., U.K.). Starved mice were deprived of food for 24h from 10:00h.

Chemicals

Materials were of the highest grade commercially available (for sources see Salmon & Hems, 1973). ^3H_2O and D-[U-^{14}C]glucose were from The Radiochemical Centre, Amersham, Bucks., U.K.

Preparation and analysis of samples

^3H_2O (2mCi) and [U-^{14}C]glucose (5μCi) were administered intraperitoneally. After 1h (unless otherwise indicated) animals were killed, tissues were rapidly frozen and homogenized in chloroform–methanol (2:1, v/v); lipids were extracted (Folch *et al.*, 1951), and the ^{3}H and ^{14}C contents of fatty acids determined, after separation of lipid classes by t.l.c. in some cases, and saponification (Salmon *et al.*, 1974). Glucose was determined by a glucose oxidase method, in whole blood removed from the heart under diethyl ether anaesthesia.

Calculation of results

The total rate of lipid synthesis in tissues was calculated from the quotient (^{3}H in fatty acid in d.p.m.)/(specific radioactivity of plasma ^3H_2O, expressed as d.p.m. per g-atom of H in total H_2O), which was converted into mol of newly synthesized fatty acid by dividing by 13.3 (Windmueller & Spaeth, 1966, 1967). The relative contribution of glucose to lipogenesis in adipose tissue, compared with liver, was inferred from the following quotient, calculated from radioactivity values expressed in d.p.m.: (^{14}C/^{3}H in adipose tissue fatty acid)/(^{14}C/^{3}H in liver fatty acid). The use of this quotient involves the reasonable assumption that circulating [^{14}C]glucose of the same specific radioactivity was available to all tissues at any one time during the course of the experiment. Absolute rates of lipogenesis from [^{14}C]glucose were not calculated, as the specific radioactivity of plasma (or tissue) [^{14}C]glucose was not determined; this parameter would be changing rapidly in the conditions of the present experiments, and no basis is available for selecting one particular instant (after the dose of [^{14}C]glucose) at which to use the measured specific radioactivity for the calculation of the lipogenic rate.

The significance of 'free' adipose tissue (rather than of total carcass fat) in fatty acid synthesis in the whole mouse was assessed as follows. Regions of free adipose tissue were dissected and weighed; the amount of ^{3}H in fatty acid was determined, and the rate of lipogenesis in each whole region was calculated. These rates are summed in Table 2. It was estimated that at least 90% of the free adipose tissue was obtained in this way in lean mice, and more in obese mice. In lean mice, this free adipose tissue (which constitutes the discrete adipose-tissue 'organ') comprised about one-half of the total body fat (weighed as solvent-extractable dry fat after saponification, in carcass excluding the head).

Results

Time-course of fatty acid synthesis

As a pre-requisite to the valid measurement of lipogenic rates, the time-course of fatty acid synthesis was determined in liver and adipose tissue (Fig. 1). The incorporation of ^{3}H from ^3H_2O into fatty acids was approximately linear with time for 90min, in both lean and obese mice. The rate of fatty acid synthesis in the liver was about twice as fast in obese mice as in lean mice.

Diurnal rhythm in fatty acid synthesis

Many reports have indicated that there are significant diurnal rhythms in metabolic parameters. Therefore this aspect of fatty acid synthesis was investigated. In both lean and obese mice, the total rate of lipogenesis was faster during the dark hours (Fig. 2). The maximum rate of fatty acid synthesis occurred between 20:00 and 24:00h in obese mice,

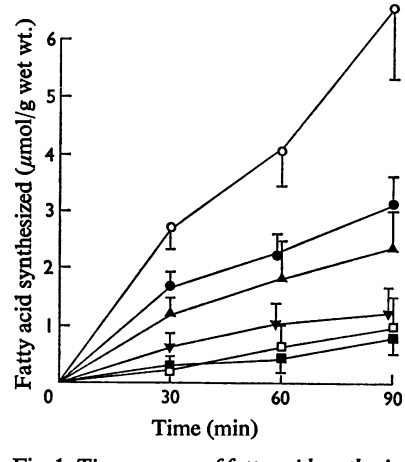

Fig. 1. *Time-courses of fatty acid synthesis*

Mice aged 3 months received ^3H_2O intraperitoneally at about 11:00h, and ^{3}H in tissue fatty acids was determined after various times. ○, □, *ob/ob* mice; ●, ■, ▼, ▲, lean mice: ●, ○, total liver lipid; ▼, liver triglyceride; ▲, liver phospholipid; ■, □, parametrial fat. Other details are in the text. Results are means of three (at 30 and 90min) or nine observations and bars indicate the S.E.M.

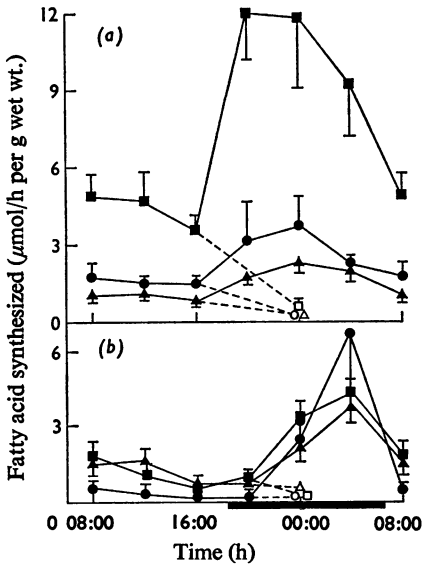

Fig. 2. *Diurnal rhythm in fatty acid synthesis*

Mice aged 6–7 weeks received ^3H_2O at various times (G.M.T.) and ^3H in tissue fatty acid was determined after 1 h, in (*a*) *ob*/*ob* mice, (*b*) lean mice: ■, liver; ●, parametrial (brown plus white) fat; ▲, scapular (brown) fat. △, ○, □, Mice deprived of food from 16:00 h. Points represent the mid-point of the experimental period (1 h). The shaded area on the abscissa indicates the dark period. Other details are in the text. Results are means of five or six observations, except for those at 08:00 h in lean mice and after food deprivation (three observations); bars indicate the S.E.M.

and at 04:00 h in lean mice. The increase in the rate of fatty acid synthesis began after 16:00 and 20:00 h in obese and lean mice respectively.

Relative $^{14}C/^3H$ ratios in adipose tissue and liver

The relative contribution of glucose to fatty acid synthesis in tissues was investigated by using a trace dose of [^{14}C]glucose (as well as ^3H_2O). The ratio of $^{14}C/^3H$ in tissue fatty acid was measured, and was always higher in adipose tissue (either parametrial or brown scapular) than in the liver of the same mice (Fig. 3), by a factor of 5–20. This factor was at its lowest between 16:00 and 04:00 h and was less (at all times) in obese mice than in lean mice. The increase in fatty acid synthesis during the night was not associated with marked changes in the blood glucose concentration in either lean or obese mice (Fig. 4).

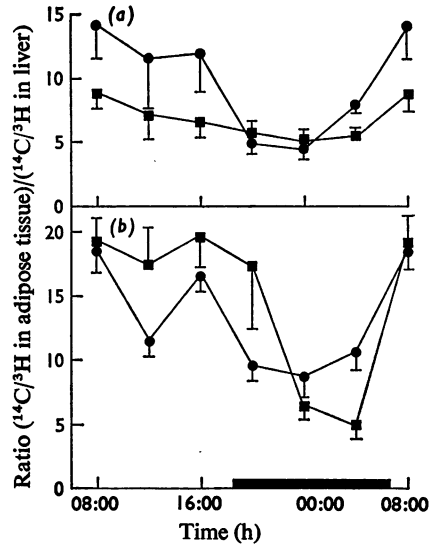

Fig. 3. *Relative $^{14}C/^3H$ ratios in adipose tissue and liver*

Mice received ^3H_2O and [U-^{14}C]glucose at various times; the experiments were those described in Fig. 2 for (*a*) *ob*/*ob* mice and (*b*) lean mice. The ratio (^{14}C in d.p.m.)/(^3H in d.p.m.) in tissue fatty acids was calculated. The $^{14}C/^3H$ ratios in adipose tissue have been divided by that in liver (in the same mice), for parametrial fat) ●) and scapular fat (■). Results are means of three to five observations. Other details are in Fig. 2 or the text.

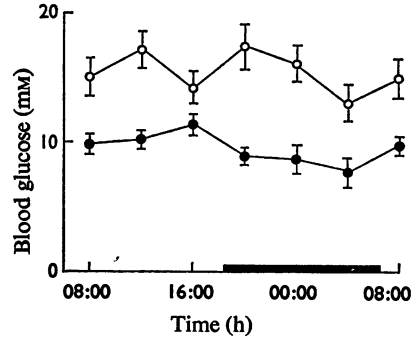

Fig. 4. *Diurnal rhythm in blood glucose concentration*

In the experiments described in Figs. 2 and 3, the glucose content of whole blood was measured at the time of killing of the *ob*/*ob* (○) or lean (●) mice. Results are means of three to five observations, and bars indicate the S.E.M.

Comparison between fatty acid synthesis rates in adipose tissue and liver

Fatty acid synthesis was measured in various adipose-tissue regions of mice (Table 1). In normal mice,

Table 1. *Synthesis of fatty acids in different regions of adipose tissue in mice*

Fed female mice aged 4–5 weeks received ^3H_2O between 10:00 and 12:00h. After 1h the amount of ^3H in fatty acid in different regions of adipose tissue was determined. Other details are in the text. Results are means±S.E.M. of the numbers of observations in parentheses, obtained from a total of six mice in each group.

	Fatty acid synthesized (μmol/g wet wt. of tissue)	
Fat region	Lean mice	*ob/ob* mice
Parametrial (white)	0.8 ±0.2 (5)	9.2±1.3 (5)†
Parametrial (brown)	2.2 ±0.9 (4)	7.4±1.0 (6)*
Mesenteric	1.2 ±0.4 (6)	7.4±0.8 (6)†
Limb subcutaneous	0.39±0.12 (6)	4.0±0.5 (5)†
Scapular (white)	0.35±0.08 (6)	3.5±0.6 (5)†
Scapular (brown)	1.8 ±0.5 (6)	2.7±0.7 (6)

* Brown areas of parametrial fat were identified by eye in lean mice; in obese mice, clearly discernible brown areas did not occur, but comparable regions adjacent to blood vessels were sampled. For these values, $P<0.02$.

† Compared with values in lean mice, $P<0.01$.

Table 2. *Relative contributions of liver and free adipose tissue to fatty acid synthesis*

Total rates of fatty acid synthesis were measured as described in Table 1, and calculated for whole mice. Some of the data are included in Fig. 1 and Tables 1 and 3. Regions of adipose tissue were dissected individually (see the Materials and Methods section); rates for each region (in mice aged 3 months) resembled in general those for mice aged 1 month (Table 1) and are not presented individually. Other details are in the text. Results are means from five mice in both groups of lean mice and from three or four in obese mice.

	1 month		3 months	
	Lean	*ob/ob*	Lean	*ob/ob*
Weight (g)				
Mouse	23.1	35.0	30.4	78.0
Liver	1.4	1.9	1.6	2.9
Adipose tissue	1.9	7.7	1.9	33.6
Fatty acid synthesis (μmol/h)				
Liver	2.9	10.8	4.2	11.6
Adipose tissue	1.4	30.3	2.3	22.0
Calculated ratio (fatty acid synthesized per mouse): (adipose tissue/liver)	0.48	2.81	0.55	1.90

rates were fastest in areas of brown fat and in mesenteric fat. In obese mice, rates were severalfold faster than in lean mice and the differences between regions were less marked.

One objective of the present experiments was to assess the relative contributions of liver and adipose tissue to fatty acid synthesis. Therefore, from measurements of fatty acid synthesis, the rates of lipogenesis per mouse in liver and free adipose tissue were calculated (Table 2). The total mass and ^3H-labelled fatty acid content of the various major regions of adipose tissue were determined after dissection (see the Materials and Methods section). The largest areas of discrete fat were in the scapular (white), mesenteric and limb-subcutaneous regions, in both lean and obese mice. These calculations show that fatty acid synthesis was more extensive in liver than in adipose tissue of normal mice, whereas the reverse was true in obese mice (Table 2).

Table 3. *Influence of starvation on fatty acid synthesis in mice*

Female mice aged 4 weeks received ^3H_2O between 10:00 and 12:00h. Liver and parametrial adipose tissue were analysed after 1h. Other details are given in the text. Results are means±S.E.M. of the numbers of observations in parentheses.

Mice	Tissue	Total fatty acid synthesized (μmol/g wet wt. of tissue)	*P* value, compared with fed control
Lean			
Fed	Liver	2.9±0.2 (5)	
Fed	Fat	3.6±1.7 (5)	
Starved (24h)	Liver	0.6±0.2 (3)	<0.01
Starved (24h)	Fat	0.9±0.2 (3)	>0.1
Ob/ob			
Fed	Liver	5.6±1.0 (5)	
Fed	Fat	6.2±1.3 (5)	
Starved (24h)	Liver	2.0±0.3 (3)	<0.1
Starved (24h)	Fat	0.7±0.1 (3)	<0.05

Influence of starvation and age on fatty acid synthesis

In genetically obese mice, hepatic lipogenesis from glucose does not decline on starvation as extensively as that in lean mice (Jansen *et al.*, 1967). Therefore the response to starvation (for 24h) of the total rate of fatty acid synthesis in the light period was investigated (Table 3). The decline in synthesis was less marked in the liver of obese mice than in lean mice, whereas that in adipose tissue fell to a rate that was lower than that in lean mice (Table 3). However, when mice were deprived of food from 16:00h and the rate of fatty acid synthesis was measured at 0:00h, the decline in hepatic fatty acid synthesis in obese mice was about as great as that in lean mice (Fig. 2); the decline in lipogenesis in adipose tissue resembled that observed after 24h starvation from 10:00h (compare Fig. 2 and Table 3). The reason for this difference in the extents of the decline in hepatic fatty acid synthesis in obese mice, on different starvation regimens, is not clear.

Table 4. *Distribution of ^3H from ^3H_2O in major lipid classes in liver of ob/ob mice*

Female *ob/ob* mice, aged 3 months, received ^3H_2O between 10:00 and 12:00h. Livers were analysed after 1h. Results are means±S.E.M. of the numbers of observations in parentheses. Other details are in the text.

Lipid fraction	Fatty acid synthesized (μmol/g wet wt. of liver)
Total fatty acid	4.0±0.4 (9)
Triglyceride fatty acid	2.8±0.6 (4)
Phospholipid fatty acid	2.1±0.5 (4)
Cholesteryl ester fatty acid	<0.1 (4)

Younger mice (aged 4 weeks) were used for the experiments described in Table 3. Comparison between these results and those in, e.g., Fig. 1 shows that the rate of lipogenesis, particularly in adipose tissue of obese mice, was higher in younger mice (expressed per g fresh wt. of tissue).

Distribution of ^3H in hepatic lipid classes

The major products of fatty acid synthesis *de novo* in the liver were triglyceride and phospholipid (Fig. 1; Table 4). In lean mice, the ^3H in phospholipid fatty acid was about twice that in triglyceride (Fig. 1). In obese mice (Table 4) the ^3H in phospholipid fatty acid was no greater than that in lean mice (compare 2.1 μmol/g in Table 4 with 1.9 after 1h in Fig. 1), whereas there was more ^3H in triglyceride fatty acid than in phospholipid (and more than in lean mice). These results resemble those obtained in intact mice with [^{14}C]glucose (Salmon & Hems, 1973) and in the perfused liver of lean mice (Salmon *et al.*, 1974).

Discussion

Diurnal rhythm in fatty acid synthesis

There is a marked diurnal rhythm in the total rate of fatty acid synthesis, in liver and adipose tissue of mice allowed free access to a starch-based diet. Both lean and obese mice ingest most of their food during the dark period (S. Jarman, unpublished work); the increased synthesis of fatty acid in this period was dependent on the availability of food, and so may be due at least partly to alterations in the pattern of circulating substrates and hormones, in response to feeding. A simple glucose effect cannot entirely explain the night-time enhancement of lipogenesis, *in vivo*, as there was no significant increase in mixed venous blood glucose concentration during this period. Insulin action is presumably involved in the activation of lipogenesis, at least in adipose tissue. The effect of ingested food (or its lack) on hepatic fatty acid synthesis in mice (see also Jansen *et al.*, 1966; Baker & Huebotter, 1972, 1973*a*,*b*) remains to be fully explained.

The present results agree with reports of increases, during the dark period of the 24h cycle in rats, in hepatic cholesterol synthesis (Edwards *et al.*, 1972) and fatty acid synthesis [measured with [^{14}C]acetate (Kimura *et al.*, 1970)]. In rats such increased rates of fatty acid synthesis in the dark period are not associated with a comparable rhythm in assayable fatty acid synthetase activity (Bruckdorfer *et al.*, 1974).

Carbon sources of newly synthesized fatty acid

Plasma glucose is clearly not a major carbon source for fatty acid synthesized in the liver, as shown by the observation that the $^{14}C/^3H$ ratio (in mice that received [^{14}C]glucose and ^3H_2O simultaneously) was consistently higher in adipose tissue than liver.

In normal mice during the light period, this ratio was higher in brown scapular fat (than in liver) by a factor of about 19. Thus the carbon contribution of glucose to hepatic lipogenesis at this time was 5% at most, in general agreement with results in the perfused mouse liver (Salmon *et al.*, 1974). In obese mice, the corresponding value was about 8%. During the light period glycogen is likely to provide significant carbon for hepatic fatty acid synthesis (Salmon *et al.*, 1974; Clark *et al.*, 1974), rather than precursors such as lactate or alanine, which do not appear to be rapidly converted into hepatic fatty acids in mice during daylight hours (Elliott *et al.*, 1974). In this 'post-absorptive' situation, the release of glucose by the liver could occur in association with lipogenesis. In obese mice both these processes are enhanced (Elliott *et al.*, 1971; and present work respectively).

During the dark period, glucose was again a minor carbon source, since the $^{14}C/^3H$ ratio was higher in adipose tissue (than in liver), by a factor of 10 in normal mice and 5 in obese mice. Thus even in this phase of enhanced lipogenesis, the maximum contribution of glucose carbon to hepatic fatty acids is about 10% in normal mice and 20% in obese mice. A role for glycogen is unlikely during the dark (feeding) period in mice, when net glycogen deposition occurs in the liver (Nowell, 1970). Therefore the major plasma source of carbon for hepatic lipogenesis in this period could consist of substrates such as lactate, which can easily generate pyruvate or acetyl residues. Lactate itself can fulfil this role in isolated liver preparations, as well as markedly enhancing fatty acid synthesis (Salmon *et al.*, 1974; Clark *et al.*, 1974). *In vivo*, such lactate would presumably be derived from the degradation of glucose by muscle or intestine. It is probable that during this phase of food ingestion, net gluconeogenesis diminishes (e.g. under insulin action); therefore fatty acid may then constitute a major fate of blood lactate (in the 'Cori cycle'). Also, hepatic fatty acids may be synthesized from

glycerol (Shreeve *et al.*, 1967), and perhaps amino acids, during the dark period.

During the light period, the $^{14}C/^{3}H$ ratio in lean mice was lower in parametrial than brown scapular fat, implying that a significant contribution of precursors other than glucose was present in parametrial fat. In obese mice, this was true for brown scapular rather than parametrial fat. These precursors remain to be identified; they could include lactate (Katz & Wals, 1974) or plasma triglyceride fatty acid synthesized *de novo* in the liver. During the dark period, the $^{14}C/^{3}H$ ratios in these two regions of adipose tissue became equal; thus glucose was contributing equally to fatty acid synthesis in both tissues, and may have been the sole carbon precursor for adipose tissue lipogenesis in this phase.

Relative roles of liver and adipose tissue in fatty acid synthesis

The present results show that fatty acid synthesis in normal mice, expressed per whole animal, is about twice as rapid in liver as in free adipose tissue (i.e. in the discrete adipose organ). Detailed calculations were made only for daylight rates (Table 2), but the increase in all rates at night shows that this conclusion obtains at all times. Re-cycling of newly synthesized fatty acid between liver and adipose tissue (or other organs) was not sufficiently rapid to invalidate this conclusion, or the $^{14}C/^{3}H$ ratios in liver and adipose tissue would have resembled each other more closely. Measurements of ^{14}C and ^{3}H in plasma fatty acid, in similar experiments, also suggest that there is not extensive release of hepatic newly synthesized fatty acid within periods as short as 1–2h (Haft, 1973).

Previous experiments with ^{14}C-labelled precursors have tended to underestimate the significance of hepatic fatty acid synthesis (e.g. Favarger, 1965), perhaps because 'isotope dilution' by other sources of acetyl-CoA (e.g. glycogen) is more extensive than in adipose tissue. For example, experiments with [^{14}C]glucose do not provide a useful measure of hepatic fatty acid synthesis, as blood glucose is of minor significance as a carbon source in mice (Salmon *et al.*, 1974; present work) or rats (Brunengraber *et al.*, 1973; Clark *et al.*, 1974).

The occurrence of rapid fatty acid synthesis in the liver need not imply that the liver has a significant role in the production of lipids stored in adipose tissue, which will depend on the proportion of newly synthesized fatty acid secreted by the liver, and taken up by adipose tissue. Definitive results on this point are not available.

During the period of enhanced lipogenesis (20:00–04:00h), the $^{14}C/^{3}H$ ratio in adipose tissue exceeded that in liver by a factor of only 9 in lean mice and 5 in obese mice. The approximate increase in the rate of fatty acid synthesis from glucose in the liver can be calculated from these values, and from the total rates of synthesis, if the reasonable presumption is made that the proportion of fatty acid synthesized from glucose in adipose tissue *in vivo* did not change markedly between the light and the dark period. This calculation suggests that the rate of conversion of glucose into fatty acid in the liver increased seven–eight-fold between the light and dark phases of lipogenesis, in normal and obese mice. Stimulation of fatty acid synthesis from glucose by feeding has been described in mice (Jansen *et al.*, 1966; Baker & Huebotter, 1972, 1973*a*,*b*). This effect is partly due to dietary glucose and its mechanism has still to be clarified.

Fatty acid synthesis in genetically obese mice

In genetically obese mice, adipose tissue is the main site of extra lipogenesis, as shown by the results with $^{3}H_2O$, despite the clear enhancement in the liver (compared with lean mice: present work; Salmon & Hems, 1973; Loten *et al.*, 1974; Shreeve *et al.*, 1967; Jansen *et al.*, 1967). This applies both in young animals before much fat has accumulated, and in older mice, as the rates of fatty acid synthesis (per whole mouse) in adipose tissue (or liver) did not change markedly between the ages of 1 and 3 months, in lean or obese mice. Thus in genetically obese mice the massive accumulation of fat that occurred during this period takes place on a relatively unchanged total complement of the metabolic apparatus involved in fat synthesis. This explains the dramatic decline, with age, in the rate of lipogenesis per g of wet adipose tissue.

The above considerations suggest that the 'insulin-resistance' of adipose tissue in obese mice, which is demonstrable in respect of lipolysis (Yen & Steinmetz, 1972) and conversion of glucose into CO_2 (Abraham & Beloff-Chain, 1971) does not preclude the massive enhancement of fatty acid synthesis in adipose tissue in the intact animal (compared with lean mice). However, the enhancement of fatty acid synthesis in adipose tissue in the dark period was less marked in obese mice. This applied both to the total rate and to the incorporation of glucose carbon, and could reflect a diminished response to the increase in plasma insulin which was likely to be implicated in the acceleration of lipogenesis.

The observed enhancement of hepatic fatty acid synthesis in obese mice, compared with lean mice, confirms previous studies with ^{14}C-labelled precursors (Jansen *et al.*, 1967; Salmon & Hems, 1973; Loten *et al.*, 1974). This could be a consequence of their increased circulating insulin concentration (Assimacopoulos-Jeannet *et al.*, 1974; Loten *et al.*, 1974; Winand *et al.*, 1968, 1969; Christophe *et al.*, 1970). In the liver of genetically obese mice, the extra fatty acid synthesized was located in triglyceride

rather than phospholipid (see also Salmon & Hems, 1973), suggesting that the enhancement of fatty acid synthesis *de novo* is not the only alteration of hepatic lipid metabolism in obese mice (Winand *et al.*, 1968, 1969). The synthesis of fatty acids *de novo* in the liver of obese mice could contribute to adipose-tissue fat storage, since the turnover of plasma triglyceride is enhanced (Salmon & Hems, 1973). The extent of any such contribution remains to be determined. From the present data approximate 'integrated' total rates of lipogenesis per 24h can be calculated. In obese mice (aged 1 month) these are about 350 μmol of fatty acid (per mouse) in the liver, and 1200 in adipose tissue. These rates are commensurate with the rate of fat deposition, even in younger obese mice, which may be as much as 0.5 g of fat/day, i.e. about 1500 μmol of fatty acid. Thus the synthesis of fatty acids in adipose tissue in particular is fast enough to account for the gain in weight in obese mice.

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