Hormonal Control of Intermediary Metabolism in Obese Hyperglycemic Mice

II. Levels of Plasma Free Fatty Acid and Immunoreactive Insulin and Liver Glycogen

R. R. Abraham, B.A., Erica Dade, M.Sc., Jennifer Elliott, B.Sc., and D. A. Hems, M.B., Ph.D., London, England

SUMMARY

In mice with genetic obesity, the plasma concentration of total free fatty acid (FFA) was not significantly raised, and no abnormality was detected in the response of the plasma level of FFA to noradrenaline. The plasma concentration of immunoreactive insulin (IRI) in the fed state was higher in obese than lean mice, even if glucose was given to lean mice. The content of liver glycogen was consistently higher in obese mice.

Following deprivation of food, plasma concentration of IRI decreased in obese mice, and that of FFA rose at the same rate as in lean mice. In obese mice which had been fasted for twenty-four hours or fed on a restricted quantity of food, the plasma level of IRI remained higher than in lean mice.

On oral administration of glucose to fasted mice, the decline in plasma FFA concentration, compared to that in animals which received saline, was similar in obese and lean mice. In response to glucose, the plasma IRI concentration increased rapidly in obese mice.

These results suggest that there is no primary impairment of adipose tissue triglyceride breakdown ("lipolysis") in genetically obese mice, and that the hypersecretion of insulin in response to ingestion of food may more closely reflect their primary disorder. DIABETES *20*:535-41, August, 1971.

In mice with genetic obesity, a decreased capacity for mobilization of total free fatty acid (FFA) from adipose tissue, especially in response to catecholamines, has been observed in isolated fat pads[1-5] and fat cells.[6,7] Such a deficiency could contribute to a tendency to obesity (for review see Hellman[8]). In the present experiments, circulating concentrations of FFA have been measured in genetically obese mice, particularly after administration of noradrenaline.

In genetically obese mice, the plasma concentration of immunoreactive insulin (IRI) increases slowly over the first few months, in association with hyperglycemia.[9] This condition of apparent "insulin resistance" has been investigated in vitro in muscle and adipose tissue (see Paper I[10]). In the experiments reported here, the relationship between the hyperinsulinemia and blood glucose concentration has been followed in vivo, in mice of the same two age groups (two to four and six to eight months).

There is rapid inhibition of fatty acid mobilization from adipose tissue in vivo during post-starvation refeeding.[11] Therefore it was of interest to measure the fall in circulating concentrations of FFA in fasted mice which received glucose. The rise in plasma level of IRI in these experiments was a measure of pancreatic β-cell response, and provided a test of the suggestion of Genuth,[12] that obese mice may secrete excess insulin in response to ingestion of glucose.

Finally, liver glycogen content has been measured in fed and fasted-refed mice, in order to compare the relationship between blood glucose and liver glycogen in lean and in obese mice.

METHODS

Animals

Obese mice, homozygous for the obese gene (*ob*), which originated in Bar Harbor, Maine, U.S.A., are referred to as *ob/ob*. Obese mice on long-term partial food deprivation ("restricted diet" mice) are designated *ob/ob-RD*. Lean mice were homozygous (ob^+/ob^+) or

From the Department of Biochemistry, Imperial College of Science and Technology, London, S.W.7, England.

heterozygous (*ob/ob*$^+$). Details of origin, diet and weights of the mice and of the feeding regimen for *ob/ob*-RD mice are given in Paper 1.[10]

Chemicals

Noradrenaline bitartrate and sodium dithiodiethyl carbamate were from Sigma Chemical Co., London. Other chemicals were of analytical grade. Bovine plasma albumin (Fraction V) was from Armour Pharmaceuticals Ltd. (Eastbourne). The insulin-binding reagent and crystalline ox insulin (23.4 U./mg.) were supplied by Burroughs Wellcome Ltd., London.

Analytical methods

Immunoreactive insulin. This was assayed, with minor modifications, by the double-antibody method of radioimmunoassay,[13] using the kit supplied by the Radiochemical Centre, Amersham, England. In the present method, the maximum counts/minute bound (in the absence of immunoreactive material) amounted to about 30 per cent of the I-125-ox insulin present in the mixture. Some plasma samples from fed *ob/ob* mice required dilution (up to tenfold) before assay, to increase the counts/minute bound to a satisfactory percentage (i.e. to enter the middle range of the assay). The sample size was 0.1 ml., and duplicate or triplicate determinations were made. I-125 was counted in a gamma-counter (Tracerlab Instruments Ltd.).

The assay curve obtained with crystalline ox insulin was not satisfactory for use as a standard curve, since it did not resemble the curves obtained with various dilutions of mouse tissue samples, i.e. plasma from *ob/ob* mice, or partially purified extracts of pancreas from lean and *ob/ob* mice. (Figure 1 shows the curve for plasma which has been superimposed on that for extracts of pancreas by assuming that immunoreactive material from each tissue behaved similarly if plasma was undiluted.) Therefore, a "standard" solution of mouse insulin was obtained as follows: pooled pancreas from ten-month-old female lean mice (approximately 2 gm.) was extracted with ethanol:H_2O:HCl (51:17:1), and a partially purified extract of insulin was obtained according to Kenny.[14] The precipitate obtained with ethanol:diethyl ether (3:5, v/v) was taken up in 0.05 NHCl, and a bioassay for insulin-like activity, by the mouse convulsion method, was kindly performed (against the 4th International insulin standard) at Burroughs-Wellcome Ltd. (Dartford, Kent). This "standard" solution contained 6.2 U./ml. of insulin (fiducial limits $\pm$ 1 U; $p = 0.95$). Samples of this solution, and of other extracts of pancreas (one each from lean and *ob/ob* mice), were assayed at various dilutions with two different batches of I-125-ox insulin (figure 1). Pancreatic extracts from lean and *ob/ob* mice gave identical dilution curves, in agreement with Genuth.[12]

An immunoassay dilution curve was not obtainable with plasma from lean mice, since not enough immunoreactive material was present. Bioassay of mouse plasma was not possible for the same reason.

Since there was no major difference in the slope of dilution curves obtained from plasma and pancreas of *ob/ob* mice (figure 1), it is a reasonable presumption that no such difference exists in lean mice. Hence the use of a "standard" curve obtained from a pancreatic extract is justified.

Results have been calculated from the percentage of maximum counts/minute bound to binding reagent, by use of the "standard" pancreatic extract curve (figure 1),

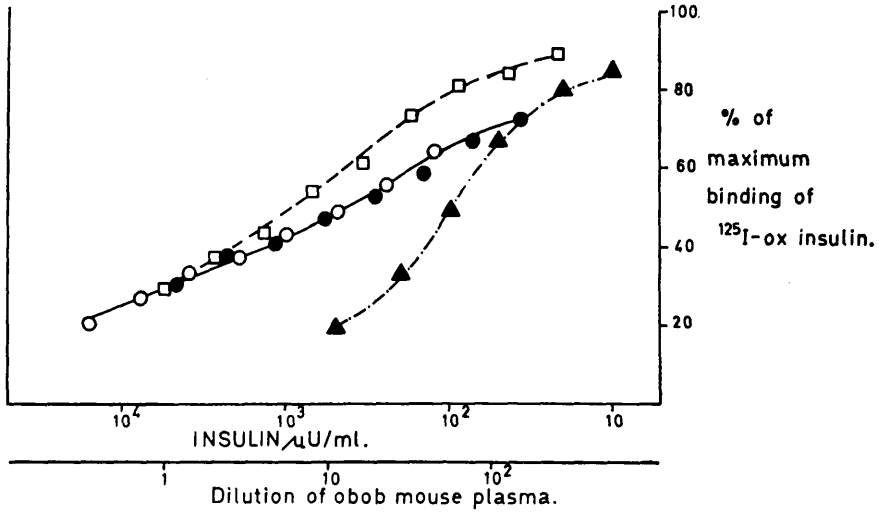

FIG. 1.
Binding of I-125-ox insulin to antibody in the presence of crystalline ox insulin (▲), different dilutions of mouse plasma (□; at 1 on the abscissa, pooled ob/ob plasma was undiluted) or pancreas extracts (●, ob/ob; ○, lean). Results are the average of two assays with different batches of I-125-ox insulin. Further details are in the text.

and expressed as μU. of pancreatic insulin.

Results from those plasma samples from *ob/ob* mice which were diluted for assay have been calculated either by obtaining a "true" percentage of counts/minute bound (i.e. that which would have been bound with an undiluted sample) by use of the plasma dilution curve (figure 1), or, if dilution was threefold or less, and if percentage counts/minute bound was 20-35 per cent, by reading directly from the "standard" pancreatic extract curve.

Free fatty acids. These were estimated by a colorimetric method which involves copper-soap formation,[15] as modified by Itaya and Ui.[16]

Glycogen and glucose. Glucose was determined by a glucose oxidase method. Blood was deproteinized with barium sulphate.[17] Glycogen was precipitated with ethanol from a suspension of liver in about 20 vols. 35 per cent KOH which was boiled for thirty minutes, hydrolyzed with amyloglucosidase from *Aspergillus niger* (kindly prepared by Professor W. J. Whelan) at pH 4.8, and determined as glucose.

Sampling of blood and liver

Experiments were begun at 10.00 hours, unless indicated in the tables. Mice were anesthetized with ether, the abdomen opened, and the heart exposed through the diaphragm. About 1-1.5 ml. blood was withdrawn into a syringe through a needle containing a negligible volume of heparin solution. Immediately afterwards, liver was removed and cooled in liquid nitrogen. Heparinized blood was centrifuged without delay. Plasma was removed and kept at 0° C.; determinations, particularly of fatty acid, were usually carried out immediately. Plasma was frozen if kept for more than a few hours.

Gastric intubation

Mice were lightly anesthetized with ether; the stomach was intubated with firm polythene tubing of about 1 mm. external bore. The required volume of glucose or saline (in controls) was injected. Mice were fully conscious within two minutes.

Determination of plasma volume

In order to facilitate the interpretation of plasma concentrations of FFA and IRI, the plasma volume of the mice was measured. A dilution method was used; I-125-labelled albumin (Radiochemical Centre, Amersham, England) was injected into the hepatic portal vein of female mice aged six months, anesthetized with Nembutal. Plasma was counted after twenty minutes. The plasma volume, expressed in ml. ± S.E.M. (number of measurements in parentheses), was 1.49 ± 0.05 (12) in lean and 1.99 ± 0.16 (5) in *ob/ob* mice.

TABLE 1

Plasma Concentrations of Free Fatty Acid in Fed and Fasted Mice

Mice aged three to four months were deprived of food from 00.00 hours for nine hours, or from 09.00 hours for zero or twenty-four hours. Results are expressed as μE/ml. plasma. Mean values are given, ± S.E.M. Number of observations in parentheses.

Mice	Sex	Duration of Starvation (hours)	Plasma FFA
Lean	Male	0	0.19 ± 0.05(5)
Lean	Female	0	0.26 ± 0.03(12)
ob/ob	Female	0	0.29 ± 0.06(6)
Lean	Female	9	0.44 ± 0.13(3)
ob/ob	Female	9	0.53 ± 0.05(4)
Lean	Male	24	0.77 ± 0.07(6)
Lean	Female	24	1.02 ± 0.10(12)
ob/ob	Female	24	0.89 ± 0.11(6)

RESULTS

Concentrations of plasma free fatty acid and immunoreactive insulin, and blood glucose in fed and fasted mice

There was no major difference in circulating FFA concentration between fed *ob/ob* and lean mice or in the time course of increase in FFA level over twenty-four hours following food deprivation (table 1).

The IRI concentration in plasma of fed *ob/ob* mice was higher than in lean mice, especially in the six to nine months age group (table 2), confirming the results reported by Westman.[9] After fasting for twenty-

TABLE 2

Concentrations of Plasma Immunoreactive Insulin and Blood Glucose in Fed and Twenty-four-hour Fasted Mice

Blood samples were obtained from mice of mixed sex. Mice which were six-hour fasted received 0.5 ml. 1 M glucose intragastrically, to achieve an initial controlled "fed" state. Results are expressed as μU./ml. plasma (IRI; units of mouse pancreatic insulin) or mg./100 ml. blood (glucose). Mean values are given ± S.E.M. Number of observations in parentheses.

Mice	Age (mo.)	Duration of Starvation (hrs.)	Plasma IRI	Blood Glucose
Lean	2-4	0	35 ± 10(9)	148 ± 7(7)
Lean	6-9	0	235 ± 45(24)	109 ± 9(16)
ob/ob	2-4	0	1,160 ± 460(11)	377 ± 58(9)
ob/ob	6-9	0	9,450 ± 2,050(12)	313 ± 35(15)
ob/ob-RD	6-9	0	2,140 ± 1,080(5)	108 ± 4(13)
Lean	2-4	6	< 35(3)	147 ± 11(6)
ob/ob	2-4	6	235 ± 60(4)	240 ± 24(6)
ob/ob-RD	2-4	6	< 35(3)	217 ± 4(3)
Lean	2-4	24	< 35(5)	81 ± 9(5)
Lean	6-9	24	< 35(7)	58 ± 7(11)
ob/ob	2-4	24	70 ± 15(6)	81 ± 11(6)
ob/ob	6-9	24	280 ± 100(6)	45 ± 6(6)
ob/ob-RD	2-4	24	< 35(3)	45 ± 6(6)

TABLE 3

Concentrations of Immunoreactive Insulin and Blood Glucose and of Liver Glycogen in Fed Mice

Fed female mice aged six to eight months received intragastric glucose. Blood was taken after sixty to ninety minutes. When three doses of glucose were given, these were at ninety-minute intervals. Results are expressed as μU./ml. plasma (IRI; units of mouse pancreatic insulin), mg./100 ml. blood (glucose) and μmoles/gm. fresh liver (glycogen). Mean values are given, ± S.E.M. Number of estimations in parentheses.

Mice	Dose of Glucose	Delay before Sampling (min.)	Plasma IRI	Blood Glucose	Liver Glycogen
Lean	1 ml. 2 M	60	270 ± 70(3)	329 ± 42(4)	—
ob/ob	1 ml. 2 M	60	> 11,000(3)	940 ± 200(3)	—
Lean	1 ml. 2 M	75	—	185(2)	145 ± 35(3)
ob/ob	1 ml. 2 M	75	—	345 ± 25(3)	705 ± 60(3)
Lean	1 ml. 2 M (three times)	90	690 ± 200(3)	261 ± 9(3)	285 ± 30(4)
ob/ob	1 ml. 2 M (three times)	90	> 16,000(3)	595 ± 30(3)	1,420 ± 45(3)

four hours, IRI concentrations in plasma had decreased, but were still higher in *ob/ob* than in lean mice (table 2).

In the above experiments, plasma IRI concentration was consistently higher in *ob/ob* mice. In an attempt to assess the significance of this hyperinsulinemia, *ob/ob*-RD mice aged six to eight months were investigated. Their plasma IRI concentration was normal, after twenty-four hours fasting (table 2). However, in the fed state, despite normal blood sugar levels, the plasma IRI was high in *ob/ob*-RD mice (table 2). Thus long-term partial food deprivation does not completely reverse the tendency to high levels of plasma IRI in *ob/ob* mice.

The plasma concentration of IRI during fasting which followed a small dose of intragastric glucose (to establish a controlled "fed" state) fell to twenty-four-hour fasted levels after six hours in lean and *ob/ob*-RD mice, but was still high (relative to other groups and to twenty-four-hour fasted levels) in *ob/ob* mice (table 2). Reduction in concentration of plasma IRI in *ob/ob* mice after fasting has been reported previously.[18]

Concentrations of blood glucose, plasma immunoreactive insulin and liver glycogen content in fed mice which received glucose

The plasma concentration of IRI did not exceed 1,000 μU./ml. plasma in lean mice after different doses of intragastric glucose, even when the blood glucose level was higher than that normally found in *ob/ob* mice, whereas it exceeded 16,000 μU./ml. in one group of *ob/ob* mice (table 3). These data are in broad agreement with those of Genuth.[12] The content of liver glycogen (in fed mice which received intragastric glucose) was consistently higher in obese animals (table 3).

Influence of noradrenaline on plasma free fatty acid concentration in fed mice

The release of fatty acids from adipose tissue of genetically obese mice in vitro exhibits decreased sensitivity to catecholamines.[1,2,4,5,7] In an attempt to detect this phenomenon in vivo, noradrenaline was administered to mice. Eight minutes after the injection of 150-200 μgm. noradrenaline per animal, plasma FFA levels were raised (compared with controls injected with saline) in both *ob/ob* and lean mice (table 4).

Breakdown of adipose tissue triglyceride in response to noradrenaline in *ob/ob* mice, although producing a "normal" rise in plasma FFA concentration, is presumably slower per unit weight of adipose tissue than in lean mice, since *ob/ob* mice contain up to ten times as much adipose tissue. Therefore it was of interest to

TABLE 4

Influence of Noradrenaline on Plasma Free Fatty Acid Concentration

Fed female mice were injected with 0.15 mg. (lean) or 0.2 mg. (obese) of noradrenaline bitartrate in 0.15 or 0.2 ml. aqueous 0.9 per cent sodium chloride subcutaneously. After eight minutes, blood was withdrawn. Results are expressed as μE/ml. plasma. Mean values are given, ± S.E.M. Number of observations is in parentheses.

Mice	Age	Treatment	Plasma FFA
Lean	4-6	Control	0.45 ± 0.10(7)
Lean	4-6	Noradrenaline	0.82 ± 0.13(10)
ob/ob	4-6	Control	0.61 ± 0.18(7)
ob/ob	4-6	Noradrenaline	1.06 ± 0.20(10)
ob/ob-RD	4-6	Control	0.27 ± 0.05(4)
ob/ob-RD	4-6	Noradrenaline	0.76 ± 0.15(4)
Lean	1-2	Control	0.47 ± 0.09(6)
Lean	1-2	Noradrenaline	0.97 ± 0.11(6)
ob/ob	1-2	Control	0.42 ± 0.07(5)
ob/ob	1-2	Noradrenaline	0.90 ± 0.10(6)

assess the response to noradrenaline of *ob/ob*-RD and young *ob/ob* mice, which are less excessively obese. The rise in plasma FFA concentration induced by noradrenaline (compared to controls which received saline) was normal in these animals (table 4).

Administration of glucose to fasted mice

When glucose was given intragastrically to twenty-four-hour fasted mice, the plasma concentration of FFA was decreased after two hours nearly to the level found in fed mice (compare tables 1 and 5). No difference between *ob/ob* and lean mice was observed in the extent of this decrease (table 5).

TABLE 5

Influence of Glucose on Plasma Concentration of Free Fatty Acid and Liver Glycogen Content in Fasted Mice

Female mice aged two to four months fasted for twenty-four hours received 1 ml. of either 0.9 per cent sodium chloride or 2 M glucose intragastrically. Blood and liver samples were taken after a further two hours. Results are expressed as μE./ml. plasma (FFA), mg./100 ml. blood (glucose) or μmoles/gm. fresh liver (glycogen). Mean values are given, ± S.E.M. Number of observations are in parentheses.

Mice	Treatment	Plasma FFA	Blood Glucose	Liver Glycogen
Lean	Saline	1.02 ± 0.15(6)	91 ± 7(7)	< 10
Lean	Glucose	0.39 ± 0.08(8)	152 ± 12(10)	131 ± 15(7)
ob/ob	Saline	1.25 ± 0.20(5)	91 ± 13(7)	< 10
ob/ob	Glucose	0.61 ± 0.16(5)	163 ± 10(6)	177 ± 15(7)

Plasma IRI concentration was measured in fasted mice which received two intragastric doses of 1 ml. M glucose. The level of IRI was 45 μU./ml. plasma in lean mice and 1,030 in *ob/ob* mice, compared to < 35 and 95 respectively in control groups which received saline (table 6). Hence the increase in concentration of IRI on refeeding in *ob/ob* mice was more rapid than in lean mice. Blood glucose concentrations were variable in this experiment (110-160 mg./100 ml. in both groups).

In order to assess whether hyperinsulinemia was secondary to massive obesity, the plasma IRI response to glucose was also measured in *ob/ob*-RD mice. The rise in plasma concentration of IRI in these mice was not much higher than that found in lean mice of the same age (table 6).

Finally the importance of the oral route of glucose administration, compared to the parenteral, was investigated by giving *ob/ob* mice two doses of subcutaneous glucose; there was a smaller rise of plasma IRI level although the rise was still greater than that in lean mice (table 6).

DISCUSSION

Fatty acid metabolism in genetically obese mice

The major source of plasma FFA is adipose tissue, and the increased concentration of FFA in the noradrenaline experiments may be regarded as due to adipose tissue triglyceride breakdown. Similarly, adipose tissue triglyceride breakdown is rapidly inhibited in glucose refeeding experiments,[11] and this inhibition may reasonably be considered as the major cause of the observed fall in FFA concentration. Hence the lack of a difference between *ob/ob* and lean mice in the response of plasma FFA to noradrenaline or glucose (since there was not a large difference in blood volume) suggests that adipose tissue triglyceride breakdown is normal in *ob/ob* mice, at least as manifested by plasma FFA concentration.

Since the *ob/ob* mouse contains up to ten times more adipose tissue than the lean mouse, the observation in vivo of "normal" rates of alteration of blood FFA concentration could imply slower rates of release of FFA per unit weight of adipose tissue. However, the absence of any defect in response to noradrenaline even in very young or "restricted diet" *ob/ob* animals (which have a lesser excess of adipose tissue) suggests that the results on the more obese animals merely reflect control of triglyceride breakdown (such as to result in normal regulation of plasma FFA concentration), rather than a deficiency of "lipolytic" capacity per unit fresh weight of tissue.

The parameter to which it would be most satisfactory to relate the "lipolytic" capacity of adipose tissue is the active cytoplasmic mass of fat cell tissue, which is difficult to estimate in the presence of excess triglyceride and of nonfat cell tissue (e.g. blood vessels and mast cells).

TABLE 6

Influence of Glucose on Plasma Level of Immunoreactive Insulin in Fasted Mice

Mice of mixed sex were fasted for twenty-four hours. Then 1 ml. 1M glucose, or 0.9 per cent sodium chloride was twice administered intragastrically, with a ninety-minute interval. After a further ninety minutes, plasma level of IRI was determined. In one experiment, glucose was administered subcutaneously (doses as above). Results are expressed as μU./ml. plasma (IRI; units of mouse pancreatic insulin). Mean values are given, ± S.E.M. Number of observations in parentheses.

Mice	Age (mo.)	Treatment	Plasma IRI
Lean	6-8	Saline	< 35(5)
Lean	6-8	Glucose	45 ± 30(5)
ob/ob	6-8	Saline	95 ± 80(4)
ob/ob	6-8	Glucose	1,030 ± 570(6)
ob/ob-RD	6-8	Glucose	78 ± 30(6)
ob/ob	6-8	Glucose (Subcutaneous)	380 ± 90(3)
ob/ob	1-2	Glucose	165 ± 90(3)

Various lines of evidence suggest that in *ob/ob* mice there may not be a great excess of active fat cell cytoplasm. For example, fat cells are fewer per unit weight of epididymal fat pad in *ob/ob* mice,[8,19] and metabolic processes seem to be proportional to cell number.[8,20] In massively obese mice, there are "cysts" of inert fat tissue.[21] Blood volume does not increase much in *ob/ob* mice, suggesting that the extra mass of tissue is not highly active metabolically. The excess weight of fat pad from *ob/ob* mice is not accompanied by a corresponding increase in total water content.[19] Measurements of nitrogen content of *ob/ob* mouse adipose tissue require careful interpretation, but again do not suggest an excess of fat cell cytoplasm in *ob/ob* mice.[1,8] Hence it is a reasonable presumption that active cytoplasmic fat cell mass is not greatly increased (per animal) in adipose or other tissues of *ob/ob* mice. Thus any major impairment of "lipolytic" capacity should have been revealed in the present experiments, especially in the fed hyperinsulinemic state, or in the less obese young *ob/ob* or *ob/ob*-RD animals.

The absence of a detectable defect in adipose tissue triglyceride breakdown in vivo in *ob/ob* mice is apparently in contradiction to the reports of such a defect in isolated adipose tissue from *ob/ob* mice, especially in response to catecholamines,[1,2,4,7] although the response to lipolytic agents, expressed per number of cells or per DNA content, was found to be relatively unimpaired in two studies.[22,23] It is possible that the large epididymal pads from *ob/ob* mice are less penetrable by catecholamines, or by the products of "lipolysis," since the fat pad preparation does not receive a supply of blood (or medium) through the normal vascular route. However, Steinmetz et al.[5] concluded that this was not a sufficient explanation for their observations. Also, the impaired response to catecholamines in isolated fat cells from *ob/ob* mice[7] is less likely to reflect diminished penetration. Thus, although increased fragility of *ob/ob* fat cells[19] could contribute to this observation, it is likely that some impairment of adipose tissue "lipolysis," especially in response to catecholamines (but not ACTH[23]), exists in isolated adipose tissue from massively obese mice. This may not be incompatible with the present results, since rates per unit weight of tissue were presumably slower in vivo (as discussed above). However, in less massively obese *ob/ob* (i.e. young or *ob/ob*-RD) mice, there was still no discernible abnormality in plasma FFA response to noradrenaline or glucose in vivo; it is possible that isolated fat cells (or pads) from such animals would behave normally. In fact, Enser,[7] and Steinmetz et al.,[5] concluded that impaired "lipolysis" in isolated adipose tissue was secondary to obesity.

Investigations in this laboratory of the turnover of blood C-14-palmitate, in vivo, show that turnover of plasma FFA is not slower in fed or fasted *ob/ob* mice (Dade and Hems, unpublished results). This finding is in accord with the presence of unimpaired "lipolysis" (especially in the absence of catecholamines) in isolated adipose tissue from fed *ob/ob* mice.[1,22,24] In view of this and the above considerations, and also the fact that impairment of adipose tissue triglyceride breakdown (including sensitivity to catecholamines) can occur secondarily to experimental "hypothalamic" obesity,[25,26] the possibility of a primary defect in this process in genetically obese mice may be regarded as unlikely.

Liver glycogen in genetically obese mice

In the resting fed state, especially after administration of glucose, the hepatic glycogen content was always higher in *ob/ob* than in lean mice. This was usually correlated with an increased blood sugar level, and is in accord with the results of Shull and Mayer,[27] who demonstrated increased turnover of liver glycogen in obese mice.

Relationship between blood concentrations of glucose and insulin

The high concentrations of plasma IRI in *ob/ob* mice which received glucose suggest that their hyperinsulinemia is due to stimulation of insulin secretion. Thus, since impaired destruction of circulating insulin does not contribute to the hyperinsulinemia,[28] the pancreatic β-cells of *ob/ob* mice are either in a state of high intrinsic sensitivity to stimulation, or are in the normal state of sensitivity, but subjected to excessive stimulation (other than by circulating glucose). This conclusion is in general agreement with that of Genuth.[12]

The presence of high plasma IRI concentrations in *ob/ob*-RD mice (despite a normal sensitivity to exogenous insulin in vivo[29,30] and in vitro[10]), and in twenty-four-hour fasted *ob/ob* mice suggest that the hyperinsulinemia of these animals may closely reflect their primary defect, and probably explains the persistence of excessive fat synthesis in adipose tissue of *ob/ob*-RD animals.[31-33] However, the partial disappearance of the excessive plasma IRI response to oral glucose in *ob/ob*-RD or young animals, the presence of excessive concentrations of blood IRI in gold-thioglucose treated animals,[18] and the fact that in the early phase of genetic obesity pancreatic insulin content is less than in lean mice,[12,18] could all suggest that the primary defect may be at a locus other than (or additional to) the process of β-cell response to ingested glucose.

ACKNOWLEDGMENT

We are grateful for advice and encouragement from Professor Sir Ernst Chain and Dr. A. Beloff-Chain. We are also indebted to Dr. G. A. Stewart and Mr. F. W. Webb of the Central Analytical Laboratories of the Wellcome Foundation for the bioassay of insulin-like activity of mouse pancreatic extract. The work was supported by the Medical Research Council, U.K., from whom R. R. Abraham and Jennifer Elliott had training scholarships.

REFERENCES

[1] Marshall, N. B., and Engel, F. L.: The influence of epinephrine and fasting on adipose tissue content and release of free fatty acids in obese-hyperglycemic and lean mice. J. Lipid Res. *1*:339-42, 1960.

[2] Leboeuf, B., Lochaya, S., Leboeuf, N., Wood, C., Jr., Mayer, J., and Cahill, G. F.: Glucose metabolism and mobilization of fatty acids by adipose tissue from obese mice. Amer. J. Physiol. *201*:19-22, 1961.

[3] Westman, S., and Hellman, B.: Release of free fatty acids from the isolated epididymal fat pad of obese hyperglycemic mice. Med. Exp. (Basel) *8*:193-99, 1963.

[4] Kamioka, T.: Studies on glucose and lipid metabolism and their related hormones in hereditarily obese-hyperglycemic mice. 3. Effects of some hormones on nonesterified fatty acid release from liver, diaphragm and epididymal adipose tissue. Folia Endocr. Jap. *41*:154-58, 1965.

[5] Steinmetz, J., Lowry, L., and Yen, T. T. T.: An analysis of the lipolysis in vitro of obese-hyperglycemic and diabetic mice. Diabetologia *5*:373-78, 1969.

[6] Mengel, V. K., and Schwabe, U.: Lipolyse an isolierten fettzellen von fettsuchtigen Mauser (obese mice). (Abstract) Arch. Pharm. Exp. Path. *260*:176, 1968.

[7] Enser, M.: Fatty acid mobilization in obese mice. Nature (London) *226*:175-77, 1970.

[8] Hellman, B.: Studies in obese-hyperglycemic mice. Ann. N.Y. Acad. Sci. *131*:541-58, 1965.

[9] Westman, S.: Development of the obese-hyperglycemic syndrome in mice. Diabetologia *4*:141-49, 1968.

[10] Abraham, R. R., and Beloff-Chain, A.: Hormonal control of intermediary metabolism in obese hyperglycemic mice. 1. The sensitivity and response to insulin in adipose tissue and muscle in vitro. Diabetes *20*:522-35, August, 1971.

[11] Baker, N., Garfinkel, A. S., and Schotz, M. C.: Hepatic triglyceride secretion in relation to lipogenesis and free fatty acid mobilization in fasted and glucose-refed rats. J. Lipid Res. *9*:1-7, 1968.

[12] Genuth, S.: Hyperinsulinism in mice with genetically determined obesity. Endocrinology *84*:386-91, 1969.

[13] Hales, C. N., and Randle, P. J.: Immunoassay of insulin with antibody precipitate. Biochem. J. *88*:137-46, 1963.

[14] Kenny, A. J.: Extractable glucagon of the human pancreas. J. Clin. Endocr. Metab. *15*:1089-95, 1955.

[15] Duncombe, W. G.: The colorimetric microdetermination of long-chain fatty acids. Biochem. J. *88*:7-10, 1963.

[16] Itaya, K., and Ui, M.: Colorimetric determination of free fatty acids in biological fluids. J. Lipid Res. *6*:16-20, 1965.

[17] Somogyi, M.: Determination of blood sugar. J. Biol. Chem. *160*:69-73, 1945.

[18] Stauffacher, W., Lambert, A. E., Vecchio, D., and Renold, A. E.: Measurements of insulin activities in pancreas and serum of mice with spontaneous ("obese" and "New Zealand obese") and induced (gold thio-glucose) obesity and hyperglycemia, with considerations on the pathogenesis of the spontaneous syndrome. Diabetologia *3*:230-37, 1967.

[19] Abraham, R. R.: Cellular characteristics of epididymal fat pads from genetically obese mice. J. Cell. Biol. In preparation.

[20] Salans, L. B., Knittle, J. L., and Hirsch, J.: The role of adipose tissue cell size and adipose tissue insulin sensitivity in the carbohydrate intolerance of human obesity. J. Clin. Invest. *47*:153-65, 1968.

[21] Hausberger, F. X.: Pathological changes in adipose tissue of obese mice. Anat. Rec. *154*:651-60, 1966.

[22] Herberg, L., Gries, F. A., and Hesse-Wortmann, Ch.: Effect of weight and cell size on hormone-induced lipolysis in New Zealand obese mice and American obese-hyperglycemic mice. Diabetologia 6:300-05, 1970.

[23] Stauffacher, W., Jeanrenaud, B., and Renold, A. E.: Metabolism du glucose dans le tissu adipeux et le muscle des animaux presentant une obesite metabolique ou une obesite par hyperphagie. Acta Clin. Belg. *23*:349, 1968.

[24] Kamioka, T.: Studies on glucose and lipid metabolism and their related hormones in hereditarily obese hyperglycemic mice. 1. Glucose metabolism and nonesterified fatty acid mobilization by isolated tissue. Folia Endocr. Jap. *41*:141-47, 1965.

[25] Haessler, H. A., and Crawford, J. D.: Fatty acid composition and metabolic activity of depot fat in experimental obesity. Amer. J. Physiol. *213*:255-61, 1967.

[26] Soyka, L. F., Haessler, H. A., and Crawford, J. D.: Altered composition and lipolysis of adipose tissue from gold thioglucose obese mice. Amer. J. Physiol. *217*:1088-93, 1969.

[27] Shull, K. H., and Mayer, J.: The turnover of liver glycogen in obese hyperglycemic mice. J. Biol. Chem. *218*:885-96, 1956.

[28] Coore, H. G., and Westman, S.: Disappearance of serum insulin in obese-hyperglycemic mice. Acta Physiol. Scand. *78*: 274, 1970.

[29] Batt, R., and Mialhe, P.: Insulin resistance of the inherently obese mouse (*ob/ob*). Nature (London) *212*:289, 1966.

[30] Chlouverakis, C., and White, P. A.: Obesity and insulin resistance in the obese hyperglycemic mouse (*ob/ob*). Metabolism *18*:998, 1969.

[31] Bates, M. W., Mayer, J., and Nauss, F.: Fat metabolism in three forms of experimental obesity. Acetate incorporation. Amer. J. Physiol. *180*:304-08, 1955.

[32] Alonso, L. G., and Maren, T.: Effect of food restriction on body composition of hereditary obese mice. Amer. J. Physiol. *183*:284-90, 1955.

[33] Hollifield, G., and Parson, W.: Body composition of mice with gold thioglucose and hereditary obesity after weight reduction. Metabolism 7:179-83, 1958.