The development of obesity in preweanling obob mice

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1. The body compositions of obob and lean (ob+ and ++) mice at 10, 12, 17 and 28 d of age were investigated using a 'cold stress' test to identify the two groups.

2. At each of these ages the obob mice were found to contain significantly more fat than the lean. At 10 d 20 % more fat was present and by 17 d the increase was 72 %. The obob mice at 28 d contained nearly three times as much fat as the lean.

3. Carcass energy was significantly higher in obob mice at all ages investigated.

4. Other changes in body composition found in the 28 d obob mice, i.e. a reduction in total carcass nitrogen and water content, were already established in the 17-d-old mice but differences at 10 and 12 d were not apparent.

5. The livers of obob mice were significantly heavier than those from lean control mice at 28 d but no differences were detected at the earlier ages.

6. The results are discussed with reference to the early origin of obesity in obob mice.

In recent years the genetically-obese (obob) mouse has become one of the most commonly used animals in obesity research. In this mutant, the infertility of the obese female and the difficulty of breeding from obese males (even when feed-restricted) necessitate the generation of obob offspring from heterozygous (ob+) parents. Obese individuals cannot be distinguished from their lean litter mates before approximately 28 d of age. Almost all studies on the obob mouse have used animals of this age, or older, with their already well-established obesity. In consequence, it has been difficult to determine whether metabolic differences between lean and obese animals are primary factors in the aetiology of the syndrome or are secondary to the massive accumulation of body fat. The absence of a test for the obob genotype has hitherto prevented work on 'pre-obese' animals from being pursued. However, tests for the obob genotype have recently been presented by Kaplan & Leveille (1974) and ourselves (Trayhurn, Thurlby & James, 1977) which are based on oxygen consumption and body temperature measurements respectively.

In the present communication we present the results of a study on the development of obesity in preweanling obob mice. The main aim of the work was to identify the age at which there was a substantial accumulation of fat in order that future studies could be made on the metabolic changes that precede obesity. An increased fat content has been found in the hind-limbs of obob mice aged 21 d by Bergen, Kaplan, Merkel & Leveille (1975). A higher total body fat in 21-d-old animals has been reported by Chlouverakis, Dade & Batt (1970) and in 17-d-old animals by Dubuc (1976). In these two studies the obob mice were not identified directly, but the increased carcass fat was inferred from the bimodal distribution in fat content in the offspring of heterozygous parents.

EXPERIMENTAL

Animals

The colony was derived from five breeding pairs of mice, known to be heterozygous for the 'ob' gene, which were obtained in 1974 from the Department of Biochemistry, Imperial College, London. These mice were derived from stock bred at the University of Aston in

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Birmingham where the 'ob' gene, originating in the Jackson Laboratory C57BL/6J strain, was introduced into the large 'Aston' strain (Bunyan, Murrell & Shah, 1976).

Food (Spillers-Spratts Rodent Breeding Diet No. 1; Spratts Patent Ltd, Barking, Essex) and water were provided *ad lib.* to the colony. The animal room was maintained at a temperature of 22·0–23·5°, and a relative humidity of 40–50% with a 12 h light–12 h dark cycle.

Identification of obob mice and selection of controls

The basis of the test for the obob genotype has been described elsewhere (Trayhurn *et al.* 1977). Litter mates derived from known heterozygous (ob+) crosses were moved from their nests to individual cages without bedding material and exposed to the 'cold' environment. The temperatures used for the various ages of mice were as follows: 4° for 17 d, 18° for 12 d and 22° for 10 d. Colonic temperature was monitored every 15 min using a fine NiCr–NiAl thermocouple probe of approximately 1 mm diameter which was inserted 10 mm into the rectum of 17-d-old animals and 4 mm into the 12- and 10-d-old animals. The thermocouple was attached to a Digitron 175 digital thermometer (Electroplan Ltd, Royston, Herts.).

Any mouse showing a decrease in colonic temperature that was obviously greater than those of its litter mates was removed (these being the obob animals) and a litter mate of the same sex and similar weight was selected as a lean control.

Preparation of carcass and organ samples

Mice were killed using diethyl ether anaesthesia, their tails cut off and the gut contents removed and weighed. Carcass weight was taken as the weight of the tail-less animal minus the weight of the gut contents, since the carcass weight after dissection was found to be a variable underestimate due to evaporative losses during the preparation of the carcass.

The organs and finely-divided carcasses were frozen with liquid nitrogen and freeze-dried for 24 h, a period shown to be adequate for drying similar samples to constant weight. Dried samples were weighed, placed in a closed container, and stored in a dessicator.

Estimation of carcass fat and defatted dry matter (DDM)

The method used was essentially that of Southgate (1971) which is based on the gravimetric analysis of material extracted with chloroform–methanol (2:1, v/v) and subsequently soluble in light petroleum (40–60° b.p.). The only modification was that the solvent was used at room temperature and the samples were homogenized twice for 2 min, each time using 50 ml solvent, followed by a final 50 ml washing.

During this procedure for the extraction of fat it is difficult to recover all the defatted material. The DDM has therefore been determined by difference from the amount of fat present in the sample.

Estimation of the N Content of the DDM

A micro-Kjeldahl method was used, the conversion of nitrogenous compounds to ammonium sulphate being achieved by boiling with concentrated sulphuric acid for 2 h in the presence of hydrogen peroxide as an oxidizing agent and selenium as a catalyst. The ammonium sulphate, after reaction with tartrate, isocyanurate and salicylate to form a coloured complex, was determined colorimetrically using an automated analytical procedure (Weber, 1973).

Carcass energy

Weighed samples of dried carcass homogenate, DDM and fat were analysed for energy content using an adiabatic bomb calorimeter (Autobomb Calorimeter CB-100; Gallenkamp and Co. Ltd, London) which had been calibrated using dry benzoic acid standards (Gallenkamp and Co. Ltd). Carcass energy was calculated by a factorial method using the weights

Table 1. *Body composition of 28-d-old lean and obob mice*

(Mean values with their standard errors for eight mice/group)

	Lean		Obob	
	Mean	SE	Mean	SE
Carcass wt (g)	17·37	1·04	19·48	0·82*
Liver wet wt (mg)	1451	119	1686	82**
Fat (g)	2·09	0·21	6·11	0·42***
DDM (g)	3·52	0·20	3·21	0·11*
Water (g)	11·76	0·68	10·17	0·31
Fat (mg/g carcass wt)	119	8	311	10***
Carcass energy (kJ)	152·1	11·4	300·1	18·2***
Carcass nitrogen (mg)	477	30	421	15*

DDM, defatted dry matter.

Values for obob mice were significantly different from those for lean (Student's paired *t* test): * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

of fat and DDM obtained for each animal and values for the energy content of the fat and DDM measured from a pooled sample for each genotype at each age. For both genotypes, at all ages, values of 38·4 and 20·4 kJ/g were used for the fat and DDM respectively.

RESULTS

In obob mice obesity became apparent on visual inspection by 28 d and Table 1 shows the body composition of mice at this age. Although the body-weight of the obob animals was slightly greater the most striking feature was the massive deposition of fat that had already occurred, with nearly three times as much fat present compared to the lean animals. This increased storage of fat was reflected in the greatly increased carcass energy, which was nearly twice that found in lean animals at this age. The other components of the body composition, the DDM and water, were both significantly reduced in weight in the obob mice by 9 and 14% respectively. Total carcass N was also significantly reduced and indicated a reduction in total body protein of approximately 12%. Liver hypertrophy, which is a feature of mature obob mice, is already evident but the increased weight of the liver from the obob mice (16%) is small at this age.

During the preweanling period obob and lean mice were studied at three different ages (10, 12 and 17 d) and the results for the body composition are presented in Table 2. Although only small differences in body-weight occurred between the two groups, significantly more fat was found in the obob mice even in animals as young as 10 d of age. At 10 d of age 20% more fat was present and at 12 d of age the difference was similar at 21%, while at 17 d of age a more substantial difference of 72% was found. In the 5 d period between 12 and 17 d of age the fat stores were expanding three times as rapidly in the obob mice compared to the lean mice. The accumulation of fat in both the lean and obob animals is shown in Fig. 1. Carcass energy also increased more rapidly during the development of the obob mice than the lean mice and this reflected the widening difference in the fat content between the two groups. At 10 and 12 d of age 12% more energy was present but by 17 d of age the difference increased to 41% additional energy in the carcasses of the obob mice.

The other abnormalities of body composition found in the 28-d-old animals, i.e. the reduction in the DDM, N and water content of the body were apparent in the 17-d-old obob animals but the changes were smaller in magnitude although statistically significant. The reductions in these three components were 4, 5 and 9% respectively. However, at 10 and

Table 2. *Body composition of preweanling lean and obob mice*

(Mean values with their standard errors for eight mice/group)

Age (d) ...	10				12				17			
	Lean		Obob		Lean		Obob		Lean		Obob	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Carcass wt (g)	6·72	0·22	6·86	0·24	7·90	0·13	7·96	0·26	10·92	0·34	11·31	0·36*
Liver wet wt (mg)	265	17	279	13	301	12	299	19	550	24	585	18
Fat (g)	0·95	0·04	1·14	0·07*	1·10	0·03	1·33	0·09*	1·59	0·11	2·73	0·21***
DDM (g)	1·05	0·05	1·03	0·04	1·33	0·03	1·30	0·04	2·08	0·05	1·99	0·06**
Water (g)	4·72	0·14	4·69	0·13	5·47	0·09	5·34	0·14	7·25	0·17	6·59	0·10***
Fat (mg/g carcass wt)	141	3	165	5*	139	3	166	6**	144	6	239	12***
Carcass energy (kJ)	57·9	2·3	64·9	3·6*	69·2	1·7	77·4	4·0*	103·3	5·4	145·5	9·2***
Carcass nitrogen (mg)	150	6	147	6	189	4	182	6	287	7	274	8*

DDM, defatted dry matter.

Values for obob mice were significantly different from those for lean mice (Student's paired t test): * $P < 0{\cdot}05$, ** $P < 0{\cdot}01$, *** $P < 0{\cdot}001$.

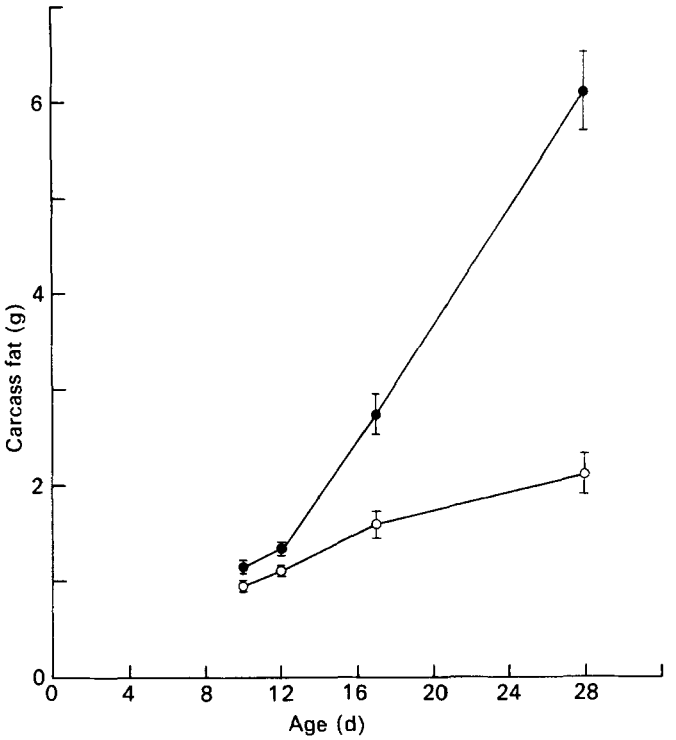

Fig. 1. Accumulation of fat (g) with age (d) in lean (○) and obob mice (●). The points are mean values with their standard errors represented by vertical bars for eight mice/group.

12 d there were no significant differences in the DDM, N, and water contents of obob and lean mice.

The liver weight of preweanling obob mice at all three ages was not increased compared to the lean mice.

DISCUSSION

The present study demonstrates that the development of obesity in obob mice has its origins early in the preweanling period. Increased fat in obob mice has been shown to be present as early as 10 d of age which is 7 d earlier than previously reported (Dubuc, 1976). Furthermore, the uncertainty inherent in other studies involving the use of a bimodal distribution of body fat as a means of separating lean and obob mice at early ages has been avoided by the direct identification of genotype. The study has also shown that a rapid expansion of the fat stores occurs in the obob mice between 12 and 17 d of age. The difference in fat content found at 17 d is very similar to that found by Dubuc (1976) who used mice with the 'ob' gene on a different genetic background to the animals used here. Joosten & Van der Kroon (1974) have also produced evidence that there is an increased rate of epididymal fat cell growth in obob mice compared with lean mice from approximately 12 d of age. With respect to the body composition of the 28-d-old animals it is noteworthy that the obob mice contained approximately three times the amount of fat present in their lean litter mates, yet at this age obesity had only just become apparent on visual inspection.

The present work has firmly established the validity and usefulness of the 'cold stress' test for identifying the obob genotype (Trayhurn *et al.* 1977). It has also shown that defective thermoregulation, which is the earliest defect that has been reported in obob mice,

cannot be regarded as a secondary consequence of obesity. Unfortunately, the ability to use the test for mice younger than 10 d of age appears to be severely limited by the absence of the capacity in lean, as well as in obese animals, to thermoregulate adequately when placed in cool environments.

The results shown in Table 2 indicated that the development of obesity preceded any abnormality of protein deposition with the implication that the changes in protein content may have developed only as a consequence of the rapid expansion of the fat stores. The precise anatomical nature of the changes in the protein and water content remain to be established but Bergen *et al.* (1975) have found the lean tissue mass and protein content to be smaller in hind-limbs from obob mice aged 21 d compared to those from lean animals, and this suggests that muscle mass is likely to be of importance in accounting for the observed change.

In conclusion, we suggest that future investigations into the aetiology of obesity in the obob mouse should be conducted, whenever possible, before the animals are weaned at 21 d of age. This should ensure that any changes observed are not secondary to the accumulation of abnormal amounts of body fat.

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