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Body Fat and Fatty Acid Synthesis in Five Lines of Mice Selected for Growth Rate[1] (37857)

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Selection of mice for body weight has demonstrated that lines of mice with widely varying mature body weights can be established (1–5). A number of studies have been conducted to determine the effect selection for growth rate has on tissue composition. Lang and Legates (5) found no change in percentage body composition in lines of mice selected for high and low 6-week weight. However, Fowler (4) observed that selection for increased 6-week body weight increased the rate of fat deposition in mice, whereas selection for low 6-week weight reduced the proportion of fat in the carcass.

Rodents have been extensively utilized as animal models in the study of obesity. While there are a number of genetically inherited forms of obesity available, in many cases afflicted animals cannot be identified until several weeks after birth (6). To study developmental aspects of obesity as well as the effect of nutritional factors on obesity, it would be desirable to have lines of mice differing in their propensity to fatten. The purpose of the study reported here was to determine body fat content and *in vitro* lipogenic capacity of liver and adipose tissue from five lines of mice selected for growth rate.

Materials and Methods. Mice. The mice used in this study were obtained from five lines of breeding stock.[2] Two lines (O_{10} and C_{10}) were originally selected from an ICR strain from the Institute of Cancer Research, Philadelphia, PA (3). The O_{10} line was selected for increased body weight gain from 21 to 42 days of age while the C_{10} line was randomly selected. Selection continued for nine generations. Three additional lines (H_6, C_2, and L_6) of mice, one selected for large body weight at 6 weeks of age (H_6), one randomly selected (C_2), and one selected for small body weight at 6 weeks of age (L_6), were originally derived by reciprocally crossing two F_1 stocks (CAF_1, $AKD2F_1$) from Jackson Laboratory, Bar Harbor, ME (5). These lines (H_6, C_2, and L_6) were selected for 30 generations. All the lines were maintained in our laboratory by random breeding within each line.

The mice were housed in plastic cages bedded with sawdust. A pelleted diet[3] and water were available *ad lib.* Litters were standardized to 7–9 animals at 4 days of age. At weaning (21 days of age), male and female mice were caged separately in groups of 10 per cage. Food intake data were not obtained.

At 7 and 14 weeks of age, two groups of 10 mice (5 males and 5 females) were randomly selected from each of the five lines. One group from each line was utilized for body-fat determinations while hepatic and adipose tissue lipogenesis and blood

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[2] Generously supplied by E. J. Eisen, Animal Science Department, North Carolina State University, Raleigh, NC.

[3] Wayne Lab Blox, Allied Mills, Inc.

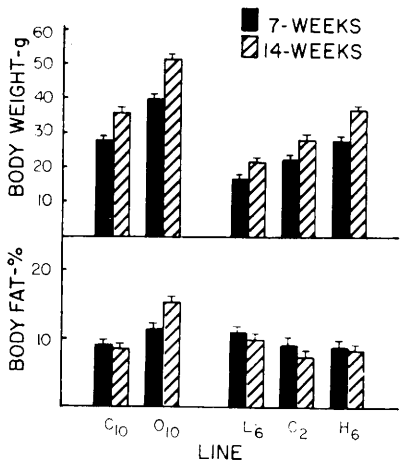

FIG. 1. Body weight and fat content of mice. Each bar represents Mean ± SEM for 10 mice (5 males and 5 females).

glucose values were obtained from the other group.

Analytical procedures. Body fat was determined as previously described (7). The entire animal was digested in a potassium hydroxide solution, saponified, and the lipid extracted and weighed.

To determine the *in vitro* rates of fatty acid synthesis, mice were decapitated, blood was collected, and the liver and epididymal (male) or parametrial (female) fat pads were rapidly removed. Livers slices were prepared with a Stadie Riggs hand microtome. Pieces of the fat pads (approximately 100 mg) and liver slices (approximately 100 mg) were incubated in a Krebs Ringer bicarbonate buffer. The buffer contained per ml: 18 mg (liver) or 4.5 mg (adipose tissue) glucose, 0.3 μCi [U-^{14}C]glucose and 0.1 U porcine insulin.[4] The incubation procedures and methods for isolating and counting radioactive fatty acids have been described (8).

Blood glucose was determined by the glucose oxidase procedure.[5]

Results. Body weight and body fat content for the five lines of mice are presented in Fig. 1. Mice selected for increased body size weighed about 10–15 g more than the corresponding control (O_{10} vs C_{10}) or small-body-size (H_6 vs L_6) mice. The O_{10} mice weighed more than 50 g at 14 weeks of age while the L_6 mice weighed about 20 g at that time.

The O_{10} mice were fatter ($P < .05$) than the C_{10} mice at both 7 and 14 weeks of age, although the differences were more pronounced at 14 weeks of age. At 14 weeks of age, O_{10} mice contained 15.5% fat while the C_{10} mice had 8.3% body fat. There were no significant differences in body fat content when the H_6, C_2, and L_6 mice were compared.

Liver weight, expressed per gram body weight, was similar for all five lines of mice (Table I). Blood glucose concentration was slightly higher in O_{10} mice than in C_{10} mice at 7 weeks of age. This difference attained statistical significance ($P < 0.05$) at 14 weeks of age. There were no significant differences in blood glucose concentrations when H_6, C_2, and L_6 mice were compared.

In vitro estimates of [U-^{14}C]glucose incorporation into fatty acids in liver and adipose tissue from the five lines of mice are presented in Table II. The rate of fatty acid synthesis in liver slices from O_{10} mice was slightly higher than that observed in slices from C_{10} mice at 7 weeks of age; however, the difference in lipogenic capacity of the liver from these mice attained a statistical significance ($P < 0.05$) at 14 weeks of age. [U-^{14}C]Glucose incorporation into fatty acids in the adipose tissue pieces was similar for the O_{10} and C_{10} mice and did not appear to be affected by the age of the animal. Rates of fatty acid synthesis in liver slices obtained from H_6, C_2, of L_6 mice were similar. Likewise, the *in vitro* lipogenic capacity of adipose tissue from these three lines of mice were similar. In contrast to the observation in O_{10} and C_{10} mice, aging decreased ($P < 0.05$) the lipogenic capacity of both liver and adipose tissue in the H_6, C_2, and L_6 mice.

Discussion. The body weights of mice used in this study agree with published values for these lines of mice (5, 9). In all

[4] The insulin used was generously supplied by Dr. R. Chance, Eli Lilly and Co., Indianapolis, IN.

[5] Glucostat, Worthington Biochemical Corporation, Freehold, NJ.

TABLE I. Liver Weight and Blood Glucose Concentration in Mice.

	Age (weeks)	Line[a]				
		C_{10}	O_{10}	L_6	C_2	H_6
Liver weight (mg/g body wt)	14	51 ± 1[b]	52 ± 2	48 ± 3	49 ± 2	52 ± 1
Blood glucose (mg/100 ml)	7	151 ± 6	161 ± 9	137 ± 4	136 ± 16	150 ± 8
	14	147 ± 3	172 ± 4	139 ± 6	142 ± 6	143 ± 7

[a] See *Materials and Methods.*

[b] Mean ± SEM for 10 mice (5 males and 5 females).

cases, males were slightly heavier than females; however, only the averages are reported (Fig. 1). Only the O_{10} mice demonstrated an increased propensity to fatten. At 14 weeks of age, the O_{10} mice had about a twofold greater percentage body fat than in the C_{10} mice. This increase in body fat content in the O_{10} mice did not account for the entire increase in body weight they exhibited. At 14 weeks of age, the nonfat body weight of the O_{10} mice was about 10 g greater than that observed in the C_{10} mice. Timon *et al.* (9) have also reported that O_{10} mice, at 8 weeks of age, were fatter than C_{10} mice. Our results also support the observation of Lang and Legates (5) that body fat content of H_6, C_2, and L_6 mice, at 8 weeks of age, were similar.

Hyperglycemia often accompanies obesity. In the present study, a moderate hyperglycemia was apparent in the 14-week-old O_{10} mice, suggesting that hyperglycemia also accompanies an increase in body fat in this line of mice.

TABLE II. *In Vitro* Conversion of [U-^{14}C] Glucose to Fatty Acids in Liver and Adipose Tissue of Mice.[a]

	Tissue			
	Liver Age (weeks)		Adipose tissue Age (weeks)	
Line[b]	7	14	7	14
C_{10}	17 ± 8[c]	36 ± 9	333 ± 26	398 ± 80
O_{10}	27 ± 8	90 ± 20	265 ± 55	352 ± 63
L_6	106 ± 18	40 ± 9	1123 ± 310	405 ± 80
C_2	108 ± 16	44 ± 8	1266 ± 210	656 ± 73
H_6	125 ± 29	35 ± 15	1142 ± 178	421 ± 85

[a] Expressed as nmoles [U-^{14}C] glucose converted to fatty acids per 100 mg tissue per 2 hr.

[b] See *Materials and Methods.*

[c] Mean ± SEM for 10 mice (5 males and 5 females).

In all five lines of mice studied, the *in vitro* rates of fatty acid synthesis were higher in the adipose tissue than in liver slices. This observation is in agreement with that of Muiruri and Leveille (10) and suggests that the relative contribution of adipose tissue to fatty acid synthesis is greater than that of the liver in these mice.

In rats and pigs, *in vitro* lipogenic capacity of adipose tissue generally decreases as the animal ages (11–13). The rate of [U-^{14}C]glucose conversion to fatty acid per 100 mg tissue was significantly ($P < 0.05$) lower at 14 weeks than at 7 weeks of age in the H_6, C_2, and L_6 mice; however, this trend was not observed in the O_{10} and C_{10} mice. The reason(s) for this strain difference is not readily apparent. Strain differences are also apparent when the relatively low rate of fatty acid synthesis in either liver or adipose tissue of O_{10} or C_{10} mice are compared with the higher rates of fatty acid synthesis in liver or adipose tissue of the H_6, C_2, or L_6 mice. An increase in adipose tissue mass occurs by hyperplasia and/or hypertrophy; the relative contribution of hyperplasia versus hypertrophy to the development of obesity varies with the strain of mice studied (14). The adipose cellular characteristics of the lines of mice used in the present study have not been established.

The O_{10} and C_{10} mice offer two lines of mice with different propensities to fatten. The relative degree of obesity in the O_{10} mice is only moderate and offers yet another animal model for the study of the obese syndrome and the metabolic defects that accompany it. While there were marked differences in body weight of the H_6, C_2, and L_6 mice, percentage body fat and rates

of fatty acid synthesis in liver and adipose tissue appear to be similar in these three lines.

Summary. Two lines of mice (O_{10} and C_{10}) were derived from an ICR strain. The O_{10} mice weighed 52 g and contained 15.5% fat while the C_{10} mice weighed 36 g and contained 8.3% fat at 14 weeks of age. At this time, the O_{10} mice exhibited hyperglycemia and an increased rate of hepatic fatty acid synthesis when compared with the C_{10} mice. Although three other lines of mice (H_6, C_2, and L_6) derived by reciprocally crossing CAF_1 and $AKD2F_1$ mice exhibited marked differences in body weight at 14 weeks of age (H_6 = 37 g; C_2 = 29 g; and L = 22 g), the percentage of body fat, blood glucose values, and *in vitro* rates of fatty acid synthesis in liver and adipose tissue were similar in these mice.

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