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Phosphorylase Kinase Deficiency and Decreased Fat Accumulation in Hybrid Male Mice (I×C3H)[1] (40996)

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Abstract. I strain mice have a deficiency of muscle phosphorylase *b* kinase. Their skeletal muscle shows a three- to fivefold elevation in glycogen concentration and a decreased rate of glycogen breakdown. In addition, I strain mice accumulate less fat with age or high fat diets than control mice. The purpose of this study was to determine whether phosphorylase *b* kinase deficiency and decreased fat accumulation of I strain mice are related. Body weight, epididymal fat pad size, adipose cell number, and size were determined in male I, C3H, and hybrid (I×C3H) mice. Since the phosphorylase kinase deficiency is sex-linked, the male offspring of the I♀×C3H♂ cross were used. As expected, phosphorylase *b* kinase activity and glycogen content in I and I×C3H skeletal muscle are similar. Body weight at 16 weeks of age in I×C3H mice resembles the body weight of I mice rather than C3H mice. Epididymal fat pad weight is not significantly different in I×C3H and I mice. Epididymal fat pad weight is significantly lower in I and I×C3H mice compared to that in C3H mice. However, adipose cell number is intermediate in I×C3H mice compared to I and C3H mice. Adipose cell size is not significantly different in the three strains. These results suggest that the decreased fat accumulation in I mice is genetically expressed with phosphorylase kinase deficiency. The inability of I mice to accumulate fat may be due to decreased adipose cell number.

I strain mice have a deficiency of muscle phosphorylase *b* kinase (1). Their skeletal muscle shows a decreased rate of glycogen breakdown (1, 2) and a three- to fivefold elevation of glycogen concentration (1). Lyon *et al.* (3) demonstrated that the gene for phosphorylase *b* kinase is sex-linked. Thus the male offspring of I♀×C57BL♂ lack phosphorylase kinase activity, while females have activity, as did both F_1 males and females from C57BL♀×I♂. However, Gross and Mayer (4), using a more sensitive assay, detected a small amount of phosphorylase kinase activity in I strain muscle (0.3% of control) and Gross *et al.* (5) also found greater than intermediate activity in heterozygous females. Additional evidence (5) suggested that the enzyme is present in I strain muscle but has an abnormal structure and consequently decreased activity.

In addition to this phosphorylase *b* kinase deficiency I strain mice also accumulate less body fat with age (6) or a high fat diet (7) than other strains of mice (A, C57BL, or C3H). The purpose of this study was to determine whether these two characteristics, phosphorylase *b* kinase deficiency, and decreased fat accumulation, of I mice are related.

Materials and methods. Male mice (I/St, C3H/St, I♀×C3H♂/St) were obtained when 4 weeks of age (L. C. Strong Research Laboratory, La Jolla, Calif.). All mice were maintained in room at 23° with a 12 hr light/12 hr dark cycle and fed *ad libitum* (Laboratory Chow, Ralston Purina Co., St. Louis, Mo.). The mice were weighed weekly and at 17 weeks of age were killed by cervical dislocation. The entire hind limb musculature of each mouse was quickly removed, trimmed of fat, rinsed in saline, and freeze clamped. Muscle was stored at −70° until assayed for glycogen and phosphorylase *b* kinase. The

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epididymal fat pads were washed on a nylon filter with saline, blotted, and weighed. Aliquots of the fat pad were used for determination of cell number and cell size according to the method of Hirsch and Gallian (8).

Glycogen was determined according to Keppler and Decker (8). The frozen leg muscle was powdered in a stainless-steel mortar at liquid nitrogen temperature, and an aliquot was weighed (120–150 mg) and added to 15% KOH in a boiling-water bath. The samples were incubated for 30 min, cooled, and then centrifuged. An aliquot of the KOH supernant was incubated with amyloglucosidase (Sigma Co. St. Louis, Mo.). The reaction was terminated with perchloric acid, centrifuged, and the supernatant was neutralized with potassium carbonate. The supernatant was assayed for glucose with glucose-6-phosphate dehydrogenase and hexokinase.

Phosphorylase *b* kinase activity was assayed by measuring the conversion of phosphorylase *b* to phosphorylase *a* (2, 10). Phosphorylase *a* was quantitated by measuring the amount of glucose-1-phosphate (GIP) formed from glycogen (11). One unit of phosphorylase *b* kinase activity converts 1 unit of phosphorylase *b* to *a* per minute and 1 unit of phosphorylase forms 1 mole of glucose-1-P/min (12).

The data were evaluated statistically by analysis of variance and comparison of means by sum of squares and the mean-square error (13).

Results. The hybrid male mice (I×C3H) of this study have a deficiency of muscle phosphorylase *b* kinase and a high muscle glycogen content which are characteristic of I mice (Table I). The growth curves for I and I×C3H male mice are similar and are lower than the growth curve for C3H male mice (Fig. 1). Even at 4 months of age body weights of I and I×C3H mice were still significantly lower than the body weight of C3H mice (Table II). The size of the epididymal fat pads of I and I×C3H were also two- to threefold less than that of C3H mice (Table II). Adipose cell size was not significantly different in the three strains of mice. However, cell number was less in I and I×C3H mice than in C3H mice. Thus, the decrease in fat pad size of I and I×C3H mice compared to the fat pad size of C3H mice was due primarily to decreased cell number. The growth curve, total weight and cell number of the epididymal fat pad of the hybrid male mice (I×C3H) resembled I mice rather than C3H mice, suggesting that the decreased fat accumulation in I mice is genetically expressed with the phosphorylase kinase.

TABLE I. PHOSPHORYLASE KINASE ACTIVITY AND GLYCOGEN CONTENT OF HIND LEG MUSCLE OF I, I × C3H, AND C3H MALE MICE

	Phosphorylase kinase (U/g)[a]	Glycogen (mg/g)
I	4 ± 1 (7)*[,b]	8.1 ± 1.0 (8)*
I × C3H	5 ± 2 (7)*	6.7 ± 0.6 (8)*
C3H	1515 ± 134 (5)†	2.2 ± 0.3 (7)†

Note. Values with different symbols (*,†) in the same column are significantly different ($P < 0.05$).

[a] Unit converts 1 unit of phosphorylase *b* to *a* per minute (8).

[b] Results are the mean ± SEM (*n*) = number of mice.

Discussion. Cahill *et al.* (14) reported that when female C3H mice are crossed with male I mice, the male hybrid Wellesley mice (C3H♀×I♂) are more like the C3H mice with the higher body weight than I mice. Thus, male hybrids with the I strain *X* chromosome have lower body weight like the I strain, but male hybrids with the C3H strain *X* chromosome (no phosphorylase

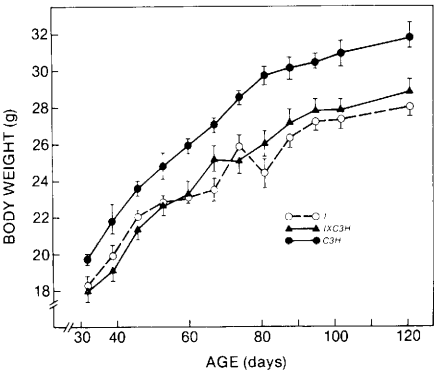

FIG. 1. Growth curve of I, I×C3H, and C3H male mice. Male mice (I/St C3H/St, and I×C3H/St) were obtained when 4 weeks of age and weighed weekly. Each point is the mean ± SEM of four to six mice.

TABLE II. FINAL BODY WEIGHT AND TOTAL EPIDIDYMAL FAT PAD WEIGHT, CELL SIZE, AND NUMBER OF I, I × C3H, AND C3H MALE MICE

	Final Body (weight (g))[a]	Total epididymal (Fat pad Wt (mg))[b]	Cell No. × 10^6	Cell size (μg lipid/cell)
I	26.9 ± 0.81 (8)*[c]	213 ± 12 (8)*	0.16 ± 0.01 (6)*	1.26 ± 0.11 (6)*
I × C3H	26.5 ± 1.23 (8)*	316 ± 50 (8)*	0.27 ± 0.03 (3)†	1.51 ± 0.26 (3)*
C3H	31.3 ± 0.70 (7)†	669 ± 47 (7)†	0.40 ± 0.05 (4)‡	1.67 ± 0.17 (4)*

Note. Values with different symbols (*,†,‡) in the same column are significantly different ($P < 0.05$).

[a] Final body weight was body weight prior to killing (cervical dislocation).

[b] Epididymal fat pads were removed, washed on a nylon filter with saline, blotted, and weighed. One aliquot of the fat pad was fixed for cell number determinations and another aliqot was extracted with chloroform:methanol for lipid determination.

[c] Values are the mean ± SEM (n) = number of mice.

kinase deficiency) have a high body weight like the C3H mice. Therefore, genetic expression of the lower body weight and decreased fat accumulation in male hybrid (I×C3H) mice is due to a gene located on the I strain X chromosome. Like *et al.* (15) did not report any differences in body weight between male and female Wellesley mice. Females would be heterozygous for the phosphorylase kinase deficiency and might show an intermediate body weight. However, Gross *et al.* (5) reported that although heterozygous female hybrid had reduced enzymatic activity, it was greater than 50% of the normal strain. This occurrence of greater than half activity in heterozygous sex-linked enzyme deficiencies is common (16). Thus, it may not be possible to distinguish females heterozygous for the I strain *X* chromosome by body weight.

The phenotypic expression of several genes (ob/ob, db/db, A^y, A^{vy}) which affect body size and fat accumulation are dependent on genetic background (17). For example, two inbred mice strains, C57BL/6J (6J) and C57BL/KsJ (KsJ), have similar body weight, blood sugar, and insulin concentration, but when bred with the ob/ob or db/db gene-marked differences in mice are observed. In the 6J-ob mice body weight is higher than in KsJ-ob, and hypertrophy of the pancreas occurs in the 6J-ob, but pancreatic atrophy occurs in KsJ-ob mice (18). The expression of the db/db gene is also more severe in KsJ mice than 6J mice (17).

The expression of the phosphorylase kinase deficiency, however, does not depend on genetic background of the mice with the defective *X* chromosome. Several substrains of I strain, I/LnJ, I/M, and I/St all have the deficiency of muscle phosphorylase kinase (4). The deficiency occurs in F_1 male hybrids of the I♀ ×C57BL♂ cross (5, 3) as well as I♀ ×C3H♂. Although the decreased fat accumulation in I mice occurs concomitantly with the phosphorylase kinase deficiency, the C3H background may have some influence on the expression of the fat accumulation in the hybrid mice (I×C3H). In the I×C3H mice the mean fat pad weight is larger than in I mice and cell number is significantly larger. Studies are in progress to study body weight and fat accumulation in other hybrids with the phosphorylase kinase deficiency.

The decreased adipose cell number in I mice observed in this study suggests I mice may have abnormal fat cell development. Cell number in mouse epididymal fat pads is established early in development and determines to a great extent the ability of an animal to accumulate fat (18). However, environmental intervention at an early age can affect the number of fat cells which develop (19). Thus, a decrease in adipose cell number in I mice may be due to either a genetic defect in adipose cell proliferation or an extrinsic effect during the critical period of development.

Faust *et al.* (21) found adipocyte number also increases in adult rats if given a highly palatable diet for 5 months. If I and C57BL mice are given a high fat diet (15%) from weanling age to 8 months of age, I strain mice gain less weight, although both strains

of mice consume the same amount of food (6). Carcass analysis of the mice indicated I mice have a lower fat content than do C56BL mice (7). I mice, in addition to a decrease in fat accumulation with a high fat diet are resistant to increases in body fat with age (6). Spontaneous activity in I and C57BL is also similar (unpublished results). Thus, the decreased cell number in I mice may be crucial to their resistance to fat accumulation and apparently the decrease in proliferation occurs at an early age and persists throughout the life of the animal.

Lyon (22) reported that I mice had normal glycogen content at 10–12 days of age but by 16–17 days of age glycogen content was elevated. Determining adipose cell number at 10 and 16 days of age might distinguish whether the decreased fat accumulation is a consequence of the phosphorylase kinase deficiency and concomitant high muscle glycogen content or due to another gene located on the *X* chromosome.

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