OBESE LOCUS IN *MUS MUSCULUS*: A GENE DOSAGE EFFECT

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The obese gene (*ob*) in *Mus musculus* was first found in the V stock of Jackson Laboratory (1) and subsequently maintained in the highly inbred C57BL/6J strain (2). Homozygous *ob*/*ob* mice, commonly named the obese-hyperglycemic mice, display syndromes resembling those of diabetes mellitus in man including hyperglycemia and obesity. Although little work has been done on the heterozygotes, numerous studies of various disciplines have compared *ob*/*ob* with ob^+/ob^+ and have been reviewed by Meier (3) and Hellman (4). Nevertheless, the primary action of *ob* is yet obscure. In studying the effect of various medium glucose concentrations on glucose oxidation by mouse epididymal adipose tissue in vitro, we found a dosage effect of *ob* which may lead to the discovery of the direct product of this gene.

MATERIALS AND METHODS

Three genotypes, namely *ob*/*ob*, ob/ob^+ and ob^+/ob^+, of Jackson C57BL/6J mice three to five months of age were used in this study. They had access to Purina laboratory chow and water until they were sacrificed by CO_2 asphyxiation. About 100 mg of tissue was incubated in Krebs-Ringer phosphate buffer, pH 7.0 (5) containing various concentrations of cold glucose and C^{14}-glucose, uniformly

labeled (New England Nuclear, about 5 m curie/m mole), in a side-arm flask. The stoppered flask containing an oxygen atmosphere was incubated 90 minutes at 37°C in a Dubnoff shaker. The tissue was then removed and homogenized for lipid extraction and protein nitrogen determination. CO_2 was collected in KOH in the center well both during incubation and after addition to the medium of H_2SO_4 from the side-arm.

The radioactivity of the KOH, a suitable diluent of the medium and the chloroform-methanol extract of the tissue lipid was measured in a toluene-based scintillation liquid using either a Nuclear Chicago Mark I or a Packard Tri-Carb 3375 counter. The protein nitrogen content of the tissue was assayed by a modification of Lowry's method (6). Blood glucose was determined on whole blood with Worthington's glucostat.

RESULTS AND DISCUSSION

The rate of glucose oxidation to CO_2 of *ob/ob* adipose tissue is much lower than that of *ob*$^+$/*ob*$^+$ tissue. This applies to all glucose concentrations tested to date, expressed either on a tissue weight basis or on a protein nitrogen basis (Fig. 1). This difference cannot be attributed to the difference in the number of fat cells in each tissue, since the *ob/ob* and *ob*$^+$/*ob*$^+$ curves are not parallel. The fact that the oxidation rate does not plateau at higher glucose concentrations is taken as evidence of a positive permeability effect by a hypertonic glucose solution. This is especially obvious at 10^{-1} M. The slower increase of *ob/ob* at concentrations beyond 2×10^{-2} M may indicate a permeability or transport defect in the *ob/ob* tissue.

It is interesting to find that the oxidation rate of *ob/ob*$^+$ tissue is intermediate to that of *ob/ob* and *ob*$^+$/*ob*$^+$ at all concentrations including the non-physiological concentration, 10^{-1} M. This gene dosage effect of *ob* on the oxidation of glucose to CO_2

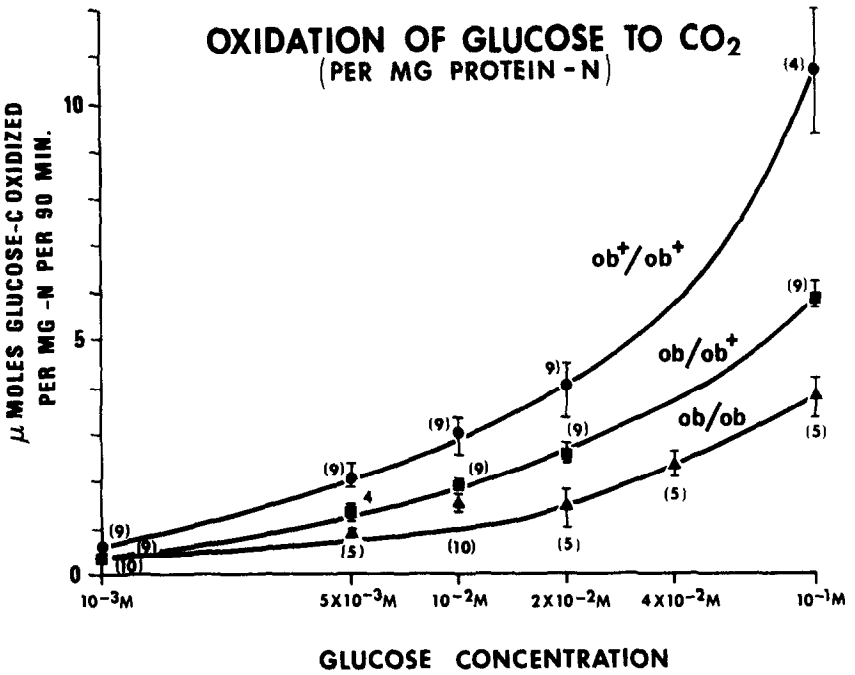

Figure 1. Relation of medium glucose concentration to the oxidation rate of glucose to CO_2 expressed on protein nitrogen basis. The number in parentheses indicates the number of mice used.

Table 1. Body Weight (g) and Blood Glucose (mg/100 ml)

	body weight (m ± s.e.)	non-fasting blood glucose (m ± s.e.)
ob^+/ob^+ (N)	28.7 ± 0.4 (68)	153.6 ± 3.3 (160)
ob/ob^+ (N)	29.6 ± 0.4 (69)	155.1 ± 3.0 (127)
ob/ob (N)	56.8 ± 0.7 (52)	361.3 ± 10.9 (114)

Table 2. Glucose (20 mM) Incorporation into lipids

ob^+/ob^+	8.76 ± 0.42	(N = 44)
ob/ob^+	8.76 ± 0.42	(N = 31)
ob/ob	4.32 ± 0.24	(N = 21)

mean ± s.e. in μmoles glucose-C/mg protein-N/90 mins.

is a specific one since in none of our other observations do we find any difference between the ob/ob$^+$ heterozygotes and ob$^+$/ob$^+$ controls. In these cases, ob/ob is always different from ob$^+$/ob$^+$ and ob/ob$^+$. Our observations include: (a) the body weight and the blood glucose level (Table 1), (b) the incorporation of glucose into lipids in adipose tissue (Table 2), (c) the in vitro response of adipose tissue to insulin as measured by glucose oxidation to CO_2 and incorporation into lipids (7), and (d) the response of the same tissue to lipolytic agents as measured by the release of free fatty acids (7).

From the dosage effect on glucose oxidation, it seems reasonable to conclude that ob is a structural gene for an entity that mediates a rate-limiting step in glucose transport or metabolism. Our data seem to eliminate the possibility that the reaction of interest is in the fatty acid synthesis pathway. Further studies are required to locate the biochemical unit coded by ob. Since the mutant form of this unit is apparently the primary etiology of the obese-hyperglycemic syndrome, its identification should greatly facilitate the study, diagnosis and treatment of this disorder.

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