The development of insulin resistance in brown adipose tissue may impair the acute cold-induced activation of thermogenesis in genetically obese (ob/ob) mice

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Genetically obese (ob/ob) mice develop insulin resistance in brown adipose tissue during the fifth week of life. Prior to this, at 26 days of age, ob/ob mice show a substantial increase in GDP binding to brown-adipose-tissue mitochondria during acute cold exposure. When insulin resistance in brown fat develops, by 35 days of age, the increase in GDP binding in response to cold is markedly reduced. Studies with 2-deoxyglucose suggest that insulin resistance in brown adipose tissue could impair thermogenic responsiveness during acute cold exposure by limiting the ability of the tissue to take up glucose.

The genetically obese (ob/ob) mouse has been widely used as an experimental animal in obesity research (1). Several recent studies have indicated that in ob/ob mice maintained at normal environmental temperatures, thermogenesis in brown adipose tissue (BAT) is abnormally low, and this is viewed as a key factor in the aetiology of obesity in the mutant (2-7). Although adult ob/ob mice adapt to chronic cold exposure by increasing the activity of the mitochondrial proton-conductance pathway (4), which is the central mechanism for thermogenesis in BAT (8), they fail to show a significant activation of the pathway on acute exposure to cold (2). In contrast, young suckling ob/ob mice can acutely activate BAT thermogenesis in the cold (5), similarly to lean mice (2). This indicates that the thermogenic unresponsiveness of BAT of the adult obese animal is a secondary defect.

Following weaning onto a standard low-fat/high-carbohydrate diet ob/ob mice exhibit a pronounced hyperlipogenesis in BAT, compared with lean animals (9). Marked hyperinsulinaemia occurs in the obese mutant at weaning, and this is likely to be the cause of the hyperlipogenesis since fatty acid synthesis in BAT is stimulated by insulin (10,11). After 26 days of age the rate of lipogenesis in BAT of ob/ob mice falls substantially, and by 35 days of age it is similar to that of lean siblings (9). This sharp decrease in lipogenesis in BAT of

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the obese during the fifth week of life occurs despite continuing hyperinsulinaemia, suggesting that there is a rapid development of insulin resistance in the tissue.

We have now examined the possibility that insulin resistance in BAT may limit thermogenic responsiveness. We show here that the activation of BAT thermogenesis in the ob/ob mouse on acute cold exposure is substantially reduced following the development of insulin resistance in the tissue, and suggest that this may relate to a requirement for glucose as a substrate during the initial response to cold.

Materials and Methods

Animals

Male lean (ob/+ or +/+) and obese (ob/ob) mice from a colony with the 'ob' gene on the mixed 'Aston' background were used. Litters were weaned onto a low-fat (3.4% w/w)/high-carbohydrate diet (LAD-1, K and K Greef Chemicals Ltd., Croydon, U.K.) at 21 days of age and group-housed in plastic cages. Food and water were available ad libitum and the mice were maintained in an animal room at 22 ± 1°C with a 12-h-light/12-h-dark cycle (light period from 0700 h). To study the effects of acute cold exposure mice were transferred for 1 h to a room at 4 ± 1°C, and caged singly in wire-mesh cages without bedding material.

Lipogenesis

Rates of lipogenesis were measured in vivo with $^3$H$_2$O as previously described (9,12). To examine the effects of insulin administration on lipogenesis, lean and obese mice were injected subcutaneously with 10 U isophane insulin/kg body weight ('Neuphane' isophane insulin injection BP, Wellcome Foundation Ltd., London, U.K.) 40 min prior to the injection of $^3$H$_2$O. This protocol produces maximal stimulation of lipogenesis in rats (11).

GDP-binding assay

The thermogenic activity of BAT was measured by the mitochondrial GDP-binding assay (13). Mitochondria were isolated (14) from BAT pooled from the interscapular and subscapular sites, and incubated at room temperature for 7 min with 10 μM ($^3$H)GDP, as previously (15). Radiochemicals were obtained from Amersham International (Amersham, U.K.). Using our assay system no nonspecific binding of GDP has been obtained with mice, nor have we observed the very-low-Kd binding site reported in hamsters (16). That the acute changes in GDP binding reported here for mice relate to functional changes in the activity of the proton-conductance pathway of BAT has been verified by other studies on acetate-induced mitochondrial swelling (manuscript in preparation).

The concentration of the 32 000-mol.wt. mitochondrial uncoupling protein from BAT, to which GDP binds, was measured by solid-phase radioimmunoassay using a mouse protein standard (17,18).