# Effects of ciglitazone on insulin resistance and thermogenic responsiveness to acute cold in brown adipose tissue of genetically obese (ob/ob) mice

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Genetically obese (ob/ob) mice develop a marked insulin resistance in brown adipose tissue soon after weaning, and this is paralleled by a fall in the acute activation of the mitochondrial proton conductance pathway in the tissue on cold exposure. Treatment of ob/ob mice with ciglitazone, a new oral hypoglycaemic, led to a restoration of insulin sensitivity in brown adipose tissue. The amelioration of insulin resistance was accompanied by a normalization of the acute, cold-induced increase in mitochondrial GDP binding. These results support the hypothesis that the development of insulin resistance in brown adipose tissue is an important factor in the impaired thermogenic responsiveness of obese mice.

*(Brown adipose tissue, Obese mouse)* *Insulin resistance* *Ciglitazone* *Lipogenesis* *GDP binding*

## 1. INTRODUCTION

The genetically obese (ob/ob) mouse has a reduced energy expenditure on brown adipose tissue (BAT) thermogenesis when maintained under normal environmental conditions, and this is now regarded as being central to the development of the obese state (review [1]). The primary mechanism for thermogenesis in BAT is a proton conductance pathway across the inner mitochondrial membrane [2]. The activity of this pathway, which is generally assessed in vitro from the binding of GDP to isolated mitochondria, is substantially reduced in ob/ob mice maintained at normal temperatures [3–5]. In addition to this abnormality, and in contrast to lean animals, adult ob/ob mice fail to exhibit an activation of the proton conductance pathway in response to acute cold exposure [3].

Recent studies have indicated that in ob/ob mice with the *ob* gene on the 'Aston' background, the defective acute cold-induced activation of thermogenesis in BAT develops during the fifth week of life [5]; in 26-day-old animals a normal response to cold occurs, but by 35 days of age the defect is evident. The defective acute activation of thermogenesis is not due to a limitation in the amount of uncoupling protein [5]. It does, however, coincide with the appearance of insulin resistance in BAT of the ob/ob mouse [5,6], which has led us to propose that insulin resistance in the tissue may be the cause of the impairment in thermogenic responsiveness, through limiting glucose uptake [5].

In the present study we have investigated the effects of treatment with an oral hypoglycaemic, ciglitazone [7–11], on the restoration of insulin sensitivity in BAT of ob/ob mice, together with the consequences for thermogenic responsiveness. The results show that ciglitazone is effective in ameliorating insulin resistance in BAT, as demonstrated by the stimulation of lipogenesis by insulin, and this is paralleled by a normalization of the activation of the proton conductance pathway on acute exposure to cold.

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## 2. MATERIALS AND METHODS

### 2.1. *Animals*

Male lean (ob/+ or +/+) and obese (ob/ob) mice of the Aston variety were taken at 30–40 days of age, and fed for 7 days on either a powdered stock diet (LAD-1, K and K Greef Chemicals, Croydon, England) alone, or powdered stock diet containing ciglitazone (1 g/kg diet). Ciglitazone, 5-[4-(1-methylcyclohexylmethoxy)benzyl]thiazolidine-2,4-dione, was a gift from Dr A.Y. Chang (Upjohn Co., Kalamazoo, USA). Both food and tap water were available ad libitum. Lean and obese littermates were housed in pairs in plastic cages containing sawdust bedding, and maintained in a room at 22 ± 1°C with a 12 h light/12 h dark cycle (lights from 07.00 h).

In studies involving the effects of acute cold exposure, the mice were transferred to a room at 4 ± 1°C for 1 h and caged individually in wire mesh cages without bedding material.

### 2.2. *Lipogenesis*

Rates of lipogenesis were measured in vivo, with $^3H_2O$ as described [6,12]. The measurements were made between 08.30 and 11.00 h. To examine the effect of insulin on lipogenesis, lean and obese mice were injected subcutaneously with 10 units isophane insulin/kg body wt ('Neuphane' isophane insulin injection BP, Wellcome Foundation Ltd, London, England) 40 min before the injection of $^3H_2O$ [5]. Control animals received a saline injection. Tissues were removed 60 min after the administration of $^3H_2O$ and processed as in [6,12].

### 2.3. *Mitochondrial GDP-binding assay*

The activity of the mitochondrial proton conductance pathway was assessed by GDP-binding studies [3,13]. BAT was removed from the interscapular and subscapular sites, which were then pooled and mitochondria isolated [13]. The mitochondria were incubated for 7 min at room temperature with 10 $\mu$M [$^3$H]GDP as described [5,13].

Radiochemicals were obtained from Amersham Internatiopnal (Amersham, England). The statistical significance of differences between groups was assessed by Student's *t*-test.

## 3. RESULTS

Table 1 shows that the administration of insulin to normal lean mice led to a substantial increase in the rate of lipogenesis in interscapular BAT. Although the 'basal' rate of lipogenesis in the interscapular pad was higher in the control obese animals than in the lean, the increase was not

Table 1

Effect of insulin on the rates of lipogenesis in brown adipose tissue of untreated or ciglitazone-treated lean and obese (ob/ob) mice

| | Lipogenesis ($\mu$gatom H incorporated/h per tissue) | | | |
|---|---|---|---|---|
| | Lean | | Obese | |
| | Control | + insulin | Control | + insulin |
| Untreated | 12.8 ± 3.7 (6) | 56.7 ± 3.7[a] (6) | 18.3 ± 2.2 (6) | 17.5 ± 1.2 (6) |
| Ciglitazone-treated | 12.1 ± 1.7 (5) | 43.5 ± 2.7[a] (5) | 24.0 ± 1.4 (6) | 44.0 ± 3.1[a] (6) |

[a] $p < 0.001$ compared with control animals of the same genotype and in the same treatment group

Lean and obese mice were given ciglitazone in the diet (1 g/kg) for 7 days. Insulin or saline (controls) was injected subcutaneously 40 min before the administration of $^3H_2O$. Interscapular brown adipose tissue was removed 60 min after the injection of $^3H_2O$. For full experimental details see text. The results are given as mean values ± SE for the number of animals shown in parentheses

statistically significant. In contrast to the lean mice, the administration of high doses of insulin to the obese animals had no effect on the rate of lipogenesis in BAT, which is in agreement with previous work on ob/ob mice of a similar age [5].

The addition of ciglitazone in the diet for 7 days had no effect on the weight gain of either lean or obese mice, nor was there any effect on the weight of the interscapular BAT pad. Glycosuria was evident in 92% (23 out of 25) of the untreated obese mice, but after treatment with ciglitazone only 11.5% (3 out of 26) of the obese animals were glycosuric (not shown).

Treatment of lean mice with ciglitazone had no effect on the basal rate of lipogenesis in BAT, and insulin produced a marked stimulation of lipogenesis in the treated lean animals. The basal rates of lipogenesis in obese mice treated with ciglitazone were higher than in the lean animals (treated or untreated), and the administration of insulin to the treated obese mice resulted in a significant stimulation of lipogenesis. The lipogenic rate in the ciglitazone-treated obese animals after the injection of insulin was similar to that in the treated lean group. Thus ciglitazone leads to the amelioration of the insulin resistance of BAT in ob/ob mice, with respect to the stimulatory effects of insulin on lipogenesis.

In the second set of experiments the activity of the mitochondrial proton conductance pathway was assessed by GDP-binding studies in untreated and ciglitazone-treated mice maintained at room temperature or exposed acutely to cold. As in the first experiments lean and obese animals were treated with ciglitazone for 7 days. The results in table 2 show that for mice maintained at 22°C GDP binding was lower in the obese than in the lean, and that treatment with ciglitazone had no significant effect on the level of binding in either genotype.

Acute exposure to cold for 1 h led to a marked increase in the level of GDP binding in the untreated lean mice, but in the untreated obese animals there was virtually no change. However, after treatment with ciglitazone the obese animals showed a substantial rise in GDP binding on cold exposure, and the rise was similar to that of the lean (treated and untreated). Thus ciglitazone normalized the acute thermogenic response to cold in ob/ob mice.

Table 2

Effect of acute cold exposure on GDP binding to brown adipose tissue mitochondria of untreated or ciglitazone-treated lean and obese (ob/ob) mice

| Treatment | Mice | GDP bound (pmol/mg mitochondrial protein) | | |
|---|---|---|---|---|
| | | 22°C | 4°C | Increase at 4°C |
| Untreated | lean | 215.0 ± 15.5 (7) | 327.1 ± 47.5[ac] (6) | 112.1 |
| Untreated | obese | 125.5 ± 25.1 (7) | 137.3 ± 11.7 (6) | 11.8 |
| Ciglitazone | lean | 231.5 ± 30.5 (7) | 355.0 ± 70.0[b] (7) | 123.5 |
| Ciglitazone | obese | 187.1 ± 30.1 (7) | 301.3 ± 32.1[ac] (7) | 114.2 |

[a] $p < 0.05$ compared with mice of the same group at 22°C
[b] $p < 0.02$, [c] $p < 0.01$ compared with the untreated obese mice at 4°C

Mice were treated with ciglitazone as in the legend to table 1. GDP binding was measured in mice maintained at 22°C, and in mice exposed to a temperature of 4°C for 1 h. The results are given as mean values ± SE with the number of animals shown in parentheses

## 4. DISCUSSION

BAT is now established as being an important site of lipogenesis in rats and mice, particularly following cold adaptation [12,14,15]. Recent studies have indicated that there is a marked hyperlipogenesis in BAT of ob/ob mice in the period immediately after weaning, and this has been attributed to the substantial hyperinsulinaemia occurring in the mutant [6] since lipogenesis in the tissue is stimulated by insulin [14,15]. During the fifth week of life there is a sharp fall in the rate of lipogenesis in BAT of ob/ob mice, despite continuing hyperinsulinaemia, indicating that the tissue becomes insulin resistant [6].

The development of insulin resistance in BAT of the ob/ob mutant occurs at the same time as an impairment in the thermogenic response to acute cold exposure, and we have suggested that there may be a causal link between the two abnormalities [5]. This proposition was examined here by investigating the effects of restoring insulin sensitivity in BAT on thermogenic responsiveness, using a new oral hypoglycaemic, ciglitazone.

Ciglitazone was selected for use in these experiments since it has recently been found to be highly effective in ameliorating insulin resistance in ob/ob mice, and other types of obese animal [7–11]. Its effects on BAT have not, however, been previously reported. Chronic treatment of ob/ob mice with ciglitazone appears to reverse successfully insulin resistance in BAT, as indicated by the restoration of the stimulatory effects of insulin on lipogenesis in the tissue.

The mechanism of action of ciglitazone is not well understood. The compound, when administered in vivo, has been found to increase the number of cell-surface insulin receptors in white adipose tissue of the ob/ob mouse [8]. However, this effect may be secondary to the reduction in the severity of hyperinsulinaemia that occurs in ciglitazone-treated ob/ob mice [7,9], since in vitro studies have suggested that the direct action of ciglitazone occurs at a step, or steps, distal to the insulin receptor [11,16].

The amelioration of insulin resistance in BAT by ciglitazone was accompanied by a normalization of the acute activation of the proton conductance pathway on cold exposure. This is clearly consistent with the suggestion that the development of an impaired response to acute cold in ob/ob mice is caused initially by insulin resistance in BAT [5]. The appearance of a thermogenic abnormality during the fifth week of life in ob/ob mice is not due to any limitation in the amount of uncoupling protein, since the concentration of the protein is normal at this stage [5,17], although there is a decline subsequently [17]. It also cannot be ascribed to a defective activation of sympathetic outflow to BAT, since noradrenaline turnover in the tissue of obese mutants is increased normally on acute cold exposure [18,19].

Insulin may play a role in the central stimulation of thermogenesis in BAT via activation of the sympathetic nervous system [20,21], but it also has direct effects on the tissue. In addition to activating pyruvate dehydrogenase and acetyl-CoA carboxylase [14,15], insulin stimulates glucose uptake by BAT [22], and the tissue may be quantitatively important in the removal of glucose from blood [22–26]. Studies with 2-deoxyglucose indicate that glucose uptake by BAT is stimulated by noradrenaline [22] and acute cold exposure [5]. Insulin resistance in BAT of ob/ob mice leads to a loss of the cold-induced increase in glucose uptake [5], and this is likely to be the explanation for the development of the impaired thermogenic response to cold. Whether glucose is required by BAT as a direct substrate during acute cold exposure [15], or as a source of oxaloacetate to support a rapid increase in fatty acid oxidation [27], is not clear.

In conclusion, although this study and our previous work [5] have focussed on the thermogenic responses to cold, it is possible that the development of insulin resistance in BAT could also be important in the impairment in diet-induced thermogenesis in obese animals.

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