Thermogenic and lipogenic activities in brown adipose tissue of I-strain mice

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The thermogenic capacity of brown adipose tissue has been investigated in I-strain mice to determine whether this tissue could play a role in the lower efficiency of food utilization reported in this strain of mice. (1) As compared with C57BL mice (a control strain), interscapular-brown-adipose-tissue weight and lipid percentage were decreased by 40% and 13% respectively in I-strain mice. (2) Mitochondrial protein content and cytochrome *c* oxidase activity were similar in the two strains, but the number of mitochondrial GDP-binding sites and uncoupling-protein content were increased by 2-fold in I-strain mice. (3) Fatty acid synthetase and citrate-cleavage enzyme (units/mg of protein) were 3-fold higher in the brown adipose tissue of I-strain mice. These results indicate that I-strain mice possess a very active brown adipose tissue. This enhanced capacity of energy dissipation in brown adipose tissue could contribute to the decreased capacity of I-strain mice to store adipose tissue.

INTRODUCTION

Mice of the I strain have a lower food efficiency, which results in a lower body fat content and gain in weight than observed in other, non-obese, strains of mice (A, C3H, C57BL) (Fenton & Carr, 1951; Fenton & Dowling, 1953). I-strain mice are also resistant to the fat accumulation that usually occurs with aging (Fenton & Dowling, 1953). This strain has therefore been considered an interesting model for the study of energy expenditure. Very recently, Hoover-Plow & Nelson (1985) reported that I-strain mice have higher O₂ consumption than have those of the C57BL (control) strain in the postprandial state and suggested that the I-strain mice might have a higher capacity for diet-induced thermogenesis.

Brown adipose tissue has been established as an important site of non-shivering thermogenesis (Foster & Frydman, 1978), and more recently Rothwell & Stock (1979, 1981) demonstrated that this tissue was also involved in diet-induced thermogenesis. Taken together, these data suggest that brown adipose tissue could play a role in the lower energy-storage efficiency observed in I-strain mice.

In the present study we investigated the thermogenic capacity of brown adipose tissue in I-strain mice as compared with C57BL mice by measuring GDP binding to mitochondria and the amount of 32 kDa protein (Ricquier & Kader, 1976) responsible for the proton-conductance pathway across the mitochondrial inner membrane (Nicholls, 1979). In addition, since fatty acids are presumed to be the main substrate for thermogenesis in brown adipose tissue and lipogenesis has been shown to be coupled with enhanced thermogenic activity (Trayhurn, 1979), we also studied the lipogenic capacity of brown adipose tissue in this strain of mice.

MATERIALS AND METHODS

Male mice (I/Crgl and C57BL/6Crgl strains) were obtained from the Cancer Research Laboratory (CRL),

University of California, Berkeley, CA, U.S.A., or from first-generation litters of parents from the CRL mated at San Diego State University. The mice were housed in a temperature-controlled room (23–25 °C) on a fixed 12 h-light (05:00–17:00 h)/12 h-dark cycle. They were fed a stock diet (Wayne Lab-Blox F6; Allied Mills, Chicago, IL, U.S.A.) *ad libitum*.

At 8 weeks of age the mice were weighed and then killed by cervical dislocation between 09:00 and 10:00 h. The interscapular brown adipose tissue was totally dissected out and carefully trimmed free of white adipose tissue. A portion of the tissue (approx. 10 mg) was used for determination of lipogenic-enzyme activities and the remainder was used for preparation of mitochondria.

Brown adipose tissues were homogenized at 0 °C in Tris (10 mM)/EDTA (2 mM)/sucrose (0.25 M) buffer, pH 7.2, and mitochondria were prepared by the method of Slinde *et al.* (1975). The activity of cytochrome oxidase was measured by a polarographic method (Schnaitman *et al.*, 1967). Specific [³H]GDP binding was assayed as previously described (Bazin *et al.*, 1984), by using the method of Nicholls (1976) as modified by Desautels *et al.* (1978). Scatchard analysis of [³H]GDP binding was performed with [³H]GDP concentrations ranging from 0.2 to 10 μM.

Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of mitochondrial proteins was carried out in a 10%-(w/v)-polyacrylamide slab gel as described in Ricquier & Kader (1976). After electrophoresis, densitometric scanning of the Coomassie Blue-stained protein was recorded at 560 nm. The relative area of the peak corresponding to the 32 kDa uncoupling protein was measured. For immunological determination, the separated mitochondrial proteins were transferred to a nitrocellulose sheet. Uncoupling protein was detected by using an antibody against rat uncoupling protein (Ricquier *et al.*, 1983) and a second antibody coupled to peroxidase (Towbin *et al.*, 1979).

For measurement of lipogenic-enzyme activities, tissue samples were homogenized (all-glass homogenizer) in

Table 1. Brown-adipose-tissue characteristics of C57BL- and I-strain mice

Brown-adipose-tissue mitochondria were prepared from 8-week-old C57BL- and I-strain mice. For experimental details, see the text. The values are means $\pm$ S.E.M. for n animals. Scatchard analysis was performed with the [3 H]GDP concentration ranging from 0.2 to 10 μ M. Three experiments were performed (six I-strain mice and six C57BL-strain mice pooled in each experiment). $P < 0.01$ or $P < 0.05$ compared with C57BL; N.S., not significant.

	C57BL strain		I strain		P
	Value	n	Value	n	
Weight (mg)	54.1 $\pm$ 1.33	43	34.6 $\pm$ 1.07	46	< 0.01
Lipid (mg)	27.7 $\pm$ 1.10	12	12.0 $\pm$ 1.06	9	< 0.01
Fat-free wt. (mg)	28.6 $\pm$ 0.79	12	21.6 $\pm$ 1.87	9	< 0.01
Mitochondrial protein (μ g/tissue)	388 $\pm$ 13.9	12	410 $\pm$ 26.8	12	N.S.
Cytochrome oxidase activity (μ mol of O ₂ /min per mg of protein)	8.7 $\pm$ 0.26	12	7.6 $\pm$ 0.51	12	N.S.
GDP binding					
(pmole/mg of protein)	169 $\pm$ 13.5	12	455 $\pm$ 66.7	12	< 0.01
(pmole/whole tissue)	65 $\pm$ 4.6	12	188 $\pm$ 28.2	12	< 0.01
Scatchard analysis of GDP binding					
Apparent dissociation constant (μ M)	0.59 $\pm$ 0.09		0.55 $\pm$ 0.08		N.S.
Maximum binding (pmole/mg)	343 $\pm$ 39		694 $\pm$ 96		< 0.05

2 ml of ice-cold 0.25 M-sucrose containing 1 mM-dithiothreitol and 1 mM-EDTA, pH 7.4. Homogenates were centrifuged at 105000 g at 0 °C for 60 min. The clear infranatants below the fat-cake were used either immediately for assays of fatty acid synthetase (Halestrap & Denton, 1973) and citrate-cleavage enzyme (EC 4.1.3.8) (Cottam & Srere, 1969) or frozen at -25 °C and used the following day for assays of 'malic' enzyme (EC 1.1.1.40), which was measured by the method of Ochoa (1955), but with 10 mM-malate. All enzyme data are expressed as units of activity; 1 unit is defined as the amount of enzyme needed to catalyse the formation of 1 nmol of product/min at 37 °C.

The protein contents of the mitochondrial preparations and of the infranatants were measured by the method of Bradford (1976).

Additional experiments were run for the determination of the lipid content of interscapular brown adipose tissue. The whole tissues were extracted with chloroform/methanol (2:1, v/v) for 24 h and lipids were determined gravimetrically.

All results were assessed for statistical significance by Student's t test.

RESULTS AND DISCUSSION

In good agreement with previous reports (Fenton & Carr, 1951; Fenton & Dowling, 1953) 8-week-old I-strain mice had a significantly lower body weight (20.4 g as against 22.6 g, $P < 0.05$) and less adipose tissue (280 mg as against 505 mg, $P < 0.01$, for inguinal + epididymal adipose tissues) than C57BL mice of the same age. Table 1 shows that interscapular-brown-adipose-tissue weight was decreased by 36% in I-strain mice compared with those of the C57BL strain. Lipid content per g of tissue weight was also decreased by more than 10% in those mice. However, brown-adipose-tissue fat-free weight was also 25% lower in I-strain mice than in the control. Thus the difference in tissue weight between the two strains of mice was not entirely due to the lower fat content.

Although the composition of brown fat was different in I- and C57BL-strain mice, mitochondrial protein content and the specific activity of cytochrome oxidase, a mitochondrial marker enzyme, were identical in the two groups of mice.

Our results (Table 1) clearly showed that, in I-strain mice, the GDP binding to brown-adipose-tissue mitochondria was dramatically increased in I-strain mice as compared with C57BL-strain mice. Scatchard analysis of GDP binding, which was performed only in three experiments, showed that the affinity of GDP for its binding site was not changed, but that maximum binding was two-fold higher in the I-strain mice (Table 1).

The specific binding of [3 H]GDP to brown-fat mitochondria has been used extensively to assess the activity of the thermogenic proton-conductance pathway. An increase in the number of binding sites would suggest that the I-strain mice have an increased concentration of uncoupling protein. However, two forms of the protein, masked inactive and unmasked active, have been postulated to exist (Desautels *et al.*, 1978; Desautels & Himms-Hagen, 1979). Recent data from Ashwell *et al.* (1985) support this hypothesis, since they have shown that, in two models of genetic obesity (*fa/fa* rat and *ob/ob* mouse) in which GDP binding was impaired from an early age (Bazin *et al.*, 1984; York *et al.*, 1984; Goodbody & Trayhurn, 1982), uncoupling protein measured by radioimmunoassay (Lean *et al.*, 1983) was not altered. In order to assess whether changes in the concentration of the uncoupling protein could account for the increased GDP binding observed in I-strain mice, we compared the proportions of uncoupling protein in the two strains after electrophoresis and immunoblotting of the mitochondrial proteins. These determinations were performed on the same mitochondria suspensions as those used for Scatchard analysis. The area of the peak corresponding to the 32 kDa protein in electrophoresed mitochondria was used as a measure of uncoupling protein (Ricquier & Kader, 1976; Mory *et al.*, 1984). It was calculated that 32 kDa protein represented 5.9% of mitochondrial

Fig. 1. Electrophoretic and immunological determinations of uncoupling protein in I-strain mice

(a, b) Densitometric tracings of Coomassie Blue-stained electrophoresed mitochondrial proteins in SDS/polyacrylamide gel from brown adipose tissue of control (a) or I-strain (b) mice. The arrow with '32' indicates the position of uncoupling protein. (c) Electrophoresed proteins were transferred to nitrocellulose and then probed with antibodies raised against uncoupling protein (Western blotting). The presence and amount of uncoupling protein were assayed by using double antibody conjugated to peroxidase. Lane 1, purified uncoupling protein; 2, mitochondria from brown adipose tissue of control mice; 3, mitochondria from brown adipose tissue of I-strain mice. This Figure illustrates the results of one typical experiment. Three experiments were performed on the same mitochondria suspensions as those used for Scatchard analysis.

Table 2. Lipogenic capacity of interscapular brown adipose tissue of C57BL- and I-strain mice

One unit is defined as the amount of enzyme needed to catalyse the formation of 1 nmol of product/min at 37 °C. The values are means $\pm$ S.E.M. for 12 C57BL- and 12 I-strain mice. $P < 0.01$ compared with C57BL.

Enzyme	Enzyme activity		Increase (fold)	P
	C57BL strain	I strain		
Fatty acid synthetase				
Units/mg of protein	77 $\pm$ 10.1	236 $\pm$ 25.4	3.1	< 0.01
Units/whole tissue	107 $\pm$ 15	421 $\pm$ 66.7	3.9	< 0.01
Citrate-cleavage enzyme				
Units/mg of protein	93 $\pm$ 9.2	286 $\pm$ 24.3	3.1	< 0.01
Units/whole tissue	131 $\pm$ 15.9	503 $\pm$ 65.3	3.8	< 0.01
'Malic' enzyme				
Units/mg of protein	101 $\pm$ 8.2	164 $\pm$ 18.5	1.6	< 0.01
Units/whole tissue	142 $\pm$ 14.8	279 $\pm$ 35	1.9	< 0.01

proteins in C57BL-strain mice and 9.8% in I-strain mice (Fig. 1). Moreover, Western blotting confirmed the increase in the level of uncoupling protein in I-strain mice (Fig. 1). [These results were corroborated by a specific radioimmunoassay of the uncoupling protein performed for us by Dr. M. Ashwell (Dunn Nutrition Laboratory, Cambridge, U.K.) on one pool of mitochondria from six mice, using the method of Lean *et al.*, 1983; a 2-fold increase in uncoupling protein was observed in I-strain mice as compared with C57BL-strain mice.] Therefore the present work demonstrates that the number of GDP-binding sites and uncoupling protein increased almost in parallel, suggesting that the increase in the number of GDP-binding sites was not due to 'unmasking'.

Our data clearly demonstrate the presence of a high thermogenic capacity in the brown adipose tissue of

I-strain mice. Furthermore, the lipogenic capacity of brown adipose tissue, as assessed by fatty acid synthetase and citrate-cleavage-enzyme activities, was 3–4-fold higher in I-strain mice than in C57BL-strain mice (Table 2). The high level of both lipogenesis and thermogenesis observed here in I-strain mice is in good agreement with the correlation between these two parameters that has been shown for several experimental models. Thus increased fatty acid synthesis in brown fat has been reported in rats and mice during cold-induced thermogenesis (McCormack & Denton, 1977; Trayhurn, 1979; Rath *et al.*, 1979) and in rats whose diet-induced thermogenesis was stimulated by glucose feeding (Sugden *et al.*, 1983). Genetically obese models, in which a very high fatty-acid-synthesis activity has been shown to co-exist in brown adipose tissue with a low GDP binding

(Lavau *et al.*, 1982; Bazin *et al.*, 1983; Mercier & Trayhurn, 1983), are noticeable exceptions to this general parallelism.

In conclusion, as compared with C57BL-strain mice, I-strain mice are characterized by a very active brown adipose tissue. The low fat content of brown fat, in spite of the high lipogenic capacity of the tissue, is likely accounted for by the large increase in mitochondrial thermogenic activity. The high thermogenic capacity of the mitochondria that we observed in this tissue could account at least in part for the lower susceptibility to obesity that characterizes these mice.

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