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Brown Adipose Tissue Thermogenesis Above the Lower Critical Temperature

M. Puerta*, I. Martín-Padura and M. Abelenda

Departamento de Fisiología Animal
Facultad de Ciencias Biológicas
Universidad Complutense de Madrid
28040 Madrid (Spain)

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Heat-acclimated rats show lighter IBAT deposit with different gross composition and lower GDP-binding than controls at thermoneutrality. A thermal disactivation of the tissue is then inferred. Cafeteria regime increased IBAT mass and GDP-binding when offered to rats at a thermoneutral ambient temperature. These results indicate that BAT thermogenesis at thermoneutrality is not the lowest one of the tissue and that diet-induced thermogenesis can take place even at thermoneutrality.

Key words: Brown adipose tissue, Thermoneutrality, Thermogenesis, Heat.

Brown adipose tissue (BAT) is the main site of nonshivering thermogenesis (5). BAT generates heat by uncoupling substrate oxidation from ATP synthesis. This process depends upon the existence of an uncoupling protein (12) also termed thermogenin (8) in the inner mitochondrial membrane.

Cold-exposition induces BAT thermogenesis and the generated heat allows the maintenance of body temperature at environmental temperatures below thermoneutrality (5). Voluntary overfeeding also induces BAT thermogenesis and the substrates ingested in excess are burned off, thus avoiding obesity (14, 15, 18).

The terms cold-induced and diet-induced thermogenesis (CIT and DIT, respectively) are commonly used to distinguish the two kinds of BAT activation mentioned above. However, the precise role of cold or diet in BAT thermogenesis is still an open question. Cold-acclimated rats show a greater food intake than normal rats and a dietary induction of BAT thermogenesis could be claimed. However, cold itself is enough to induce BAT thermogenesis since food restriction does not inhibit it in chronically cold-exposed rats (7, 13). However it is not known whether overfeeding itself can activate BAT since previous works demonstrating DIT have been carried out in mild cold (15); thus, a thermal induction may also be claimed. At thermoneutrality, there is not CIT and this environmental range is suitable to study dietary effects on BAT thermogenesis. Accordingly, this study was undertaken to determine whether vol-

* To whom all correspondence should be addressed.

untary overfeeding itself induces BAT thermogenesis. On the other hand, it is not known whether BAT obligatory thermogenesis (both CIT and DIT being absent) remains unchanged in heat or if it is depressed due to the external heat load, which led us to also study BAT thermogenesis upon chronic heat exposure.

Materials and Methods

Three groups of female Wistar rats, 180 g b.w., were used. In the first group, rats were fed with a conventional laboratory diet *ad libitum* at 28° C. This group was used as control. A second group of animals was also fed *ad libitum* but maintained at 32° C. The third group was maintained at 28° C but they were offered a cafeteria diet. All groups were maintained at the corresponding ambient temperature during 2 weeks before the experimental period which lasted 3 more weeks. The conventional diet was a commercial one (Panlab, Spain) containing (%): 66.7 carbohydrate, 19.3 protein, 3.4 fat, 4.9 cellulose and 5.7 minerals and a caloric density of 2900 Kcal/kg. The cafeteria fed animals were offered two different palatable food items daily in addition to their normal conventional diet, the choice of food being altered daily (chocolate, cheese, cookies, condensed milk, sausage). All rats were in individual cages with water *ad libitum* and a light-dark period 12:12.

At the end of the experiment all rats were sacrificed by decapitation. Interscapular brown adipose tissue (IBAT) was quickly removed and placed in an ice-cold medium containing 250 mM sucrose, 5 mM TES, 2 mM EDTA, pH 7.2. After removal of adhering white adipose tissue and muscle, IBAT was weighed and either stored at -20° C until analysis of total lipids and nucleic acids or processed to isolate mitochondria according to CANNON and LINDBERG (2) with minor modifications. In brief, 1 g of IBAT (pooled from 3-4 animals) was minced with scissors dispersed in the above cited medium to 5 % (w/v) and homogenized. The homogenate was filtered through a gauze and centrifuged at 8,500 g for 20 min, the supernatant was discharged and the walls of the tube were cleaned to remove adhering fat. The pellet was resuspended in 5 ml of fresh medium containing 80 µM free fatty acid albumin, transferred to a clean tube and diluted to the original volume with the same albumin-medium. The suspension was centrifuged at 700 g for 10 min. The supernatant was centrifuged at 8500 g for 15 min to sediment mitochondria. The pellet was resuspended with fresh albumin-medium and centrifuged again. The mitochondrial pellet was resuspended in 0.5 ml of fresh medium without albumin. All steps were carried out at 0-4° C. Cytochrome oxidase activity and total proteins were measured both in the homogenate and in the mitochondrial suspension to estimate mitochondrial recovery, that ranged 15-20 %. Cytochrome oxidase activity and protein were determined following the YONETANI and RAY (19) and LOWRY *et al.* (9) procedures, respectively.

GDP-binding was estimated as described by NEDERGAARD *et al.* (11) with some modifications. Mitochondria were incubated in 2 ml of a medium containing (final concentrations): 100 mM sucrose (0.2 µCi ^{14}C-sucrose), 20 mM TES, 1 mM EDTA, 5 µM rotenone, 10 µM GDP (0.27 µCi ^{3}H-GDP) pH 7.1. Mitochondria were added to give a final concentration of 0.5 mg/ml of mitochondrial protein. After 7 min of incubation at 25° C under agitation, 0.4 ml of the incubation were rapidly filtered by water-pump suction through Sartorius cellulose membrane filter with a pore size of 0.45 µm. The filters were placed in scintillation vials containing Triton X-100, toluene (1:2) and 4.2 g/l of PPO. Once the membranes were desintegrated, ^{3}H and ^{14}C was measured in a scintillation counter. The

amount of GDP-binding was calculated as the excess amount of (^{3}H)-GDP found in the filter by correcting for trapped buffer by the use of ^{14}C-sucrose as extramitochondrial marker.

Total lipids were extracted from IBAT according to FOLCH *et al.* (4) and were weighed after evaporating the chloroformic phase by vacuum. Nucleic acids were extracted and measured after SCHNEIDER's procedure (16).

Comparison between groups was done by an umpaired Student's t-test.

Results and Discussion

It is well known that BAT activation occurs in cold and during overfeeding at room temperature, but the role of BAT in the absence of cold-stimulation is less precisely defined. It could be speculated that BAT thermogenic activity would remain constant from the lower critical temperature onward since its facultative heat production would be unnecessary above thermoneutrality. Our contention is that such speculation needs to be presented.

In the present experiment both cold-induced ant diet-induced thermogenesis were absent in control rats since they were conventionally fed (DIT absent) at 28° C, their lowest critical temperature (CIT absent) (table I). Therefore, IBAT mass and composition of control rats were taken as indexes of facultative thermogenic inactivity. The heat-acclimated rats showed a well-defined decrease in IBAT mass, protein content and GDP-binding (table I). Nucleic acid contents and cytochrome oxidase activity were also smaller, although their values did not reach statistical significance, whereas lipid contents remained unchanged. These changes are opposite those taking place in cold acclimation, namely, increased IBAT mass, higher protein and nucleic acid contents, mitochondrial proliferation and increased GDP-binding and decreased lipid content (11, 13, 17). By comparing our results with those cited above, a reduction in BAT activity upon heat acclimation appears to be evident. This agrees with the

Table I. *Environmental temperature and dietary effects on rat brown adipose tissue.*
Values are mean ± SE of the numbers of animals given in parentheses except for cytochrome oxidase activity and GDP-binding where 3 pools fo 3-4 animals were measured in duplicate or triplicate.

	28 °C		32 °C		28 °C + Caf	
Body weight gain (g)	40.17 ± 1.78	(15)	32.90 ± 1.72	(17)**	56.68 ± 2.30	(14)**
IBAT (mg)	297.99 ± 13.17	(15)	236.29 ± 9.77	(17)**	404.44 ± 15.84	(14)**
IBAT/B.W. (%)	0.136 ± 0.005	(15)	0.117 ± 0.004	(17)**	0.179 ± 0.007	(14)**
IBAT composition						
Protein (%)	4.92 ± 0.30	(6)	2.97 ± 0.24	(6)**	6.77 ± 0.35	(5)**
RNA (%)	0.024 ± 0.004	(5)	0.020 ± 0.004	(5)	0.175 ± 0.014	(5)**
Lipids (%)	56.01 ± 2.35	(6)	58.49 ± 2.44	(5)	54.96 ± 1.54	(5)
DNA (%)	0.088 ± 0.003	(6)	0.064 ± 0.015	(6)	0.076 ± 0.003	(5)
Mitochondrial proteins (mg)	5.97 ± 0.66	(9)	6.88 ± 0.34	(9)	8.73 ± 0.91	(9)*
Cytochrome oxidase activity (μmol oxidized. min^{-1})	18.63 ± 1.17	(6)	14.62 ± 1.74	(8)	35.63 ± 2.45	(6)**
GDP-binding						
nmol per IBAT depot	1.75 ± 0.35	(9)	0.77 ± 0.08	(9)*	4.50 ± 0.28	(9)**
nmol/mg mit protein	0.29 ± 0.03	(9)	0.11 ± 0.01	(9)**	0.52 ± 0.02	(9)**

* $P<0.05$; ** $P<0.01$ when compared with conventionally fed rats at 28 °C.

reduced metabolic rate observed in mammals upon heat acclimation (1, 3). From the results shown it is worthnoting that BAT metabolic activity at thermoneutrality is not the lowest of this tissue since it can be depressed by heat exposure. On the other hand, it is also evident that the level of uncoupled oxidation taking place at thermoneutrality is not the lowest one occurring in BAT. In fact, our mitochondrial GDP-binding values for acclimated rats at 32° C are clearly lower than controls at thermoneutrality.

Cafeteria fed animals at 28° C showed, when compared to controls, a greater IBAT mass, higher protein and nucleic acid contents, higher oxidative capacity and increased GDP-binding (table I). These changes are similar to those obtained with cafeteria feeding at room temperature (6) and with cold acclimation (10) and they are indicative of a BAT thermogenic state. Such results reveal that BAT thermogenesis can be initiated only with dietary signals and that temperatures below the thermoneutral zone are not necessary for activating BAT. KUROSHIMA and YAHATA (7) and PUERTA and ABELENDA (13) have already shown that cold can induce NST in BAT of rats pair-fed with controls at thermoneutrality. Now, the environmental temperatures below thermoneutrality can be excluded as a necessary condition for diet-induced thermogenesis.

In summary, the present results reveal that BAT thermogenesis at thermoneutrality is not the lowest thermogenic activity that can be displayed by this tissue. Indeed, it is depressed by heat-acclimation. Our results further reveal that BAT thermogenic activity at thermoneutrality is no unequivocally constant since it increases with hyperphagia despite the fact that, at thermoneutrality, the organism does not need the facultative heat generated by BAT for body temperature maintenance.

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Resumen

Los depósitos de grasa parda de los animales aclimatados al calor son menores y con diferente composición que los de los controles mantenidos en la zona termoneutra. También presentan una menor unión de GDP. Sin embargo, la alimentación de ratas mantenidas en la zona termoneutra con dieta de cafetería todavía induce una hipertrofia del tejido, así como un incremento en la unión de GDP. De estos resultados se deduce, en primer lugar, que la termogénesis que presenta la grasa parda en animales mantenidos en la zona termoneutra no es nula, puesto que puede disminuir con la exposición al calor y, en segundo, que la grasa parda puede activarse termogénicamente incluso en la zona termoneutra, puesto que lo hace en animales sobrealimentados mediante dieta de cafetería.

Palabras clave: Tejido adiposo pardo, Termoneutralidad, Termogénesis, Calor.

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