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Evidence for masking of brown adipose tissue mitochondrial GDP-binding sites in response to fasting in rats made obese by dietary manipulation

Effects of reversion to standard diet

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A specific immunoassay of uncoupling protein (UCP) and measurement of GDP binding were used to study the chronic responses of brown adipose tissue (BAT) mitochondria from rats made obese by dietary means (cafeteria rats) and from obese rats subsequently fed a standard diet (post-cafeteria rats). We studied the response to fasting in order to assess the masking/unmasking responses in these groups. These studies have shown the following. (1) In the obese rats (cafeteria and post-cafeteria) the chronic increase in mitochondrial UCP concentration compared with controls parallels the increase in GDP binding. (2) In 24 h-fasted control rats the decrease in GDP binding is associated with a change in UCP concentration, but in fasting cafeteria and post-cafeteria obese rats the decrease in GDP binding is not associated with any change in UCP concentration. (3) Post-cafeteria obese rats showed increased GDP binding and higher UCP concentrations than the controls, but these values were less than in cafeteria obese rats. (4) Control rats at 8 months old showed greater GDP binding and had a higher UCP concentration than 11-month-old control rats. (5) The responses of GDP binding and UCP concentration to fasting in post-cafeteria obese rats were similar to those in cafeteria obese rats, suggesting that such abbreviations are related to the obese status itself rather than to the composition of the cafeteria diet. The evidence supports the hypothesis that the response of the cafeteria and post-cafeteria obese rats to fasting is associated with a masking of UCP, whereas with chronic manipulation of diet changes in UCP concentration predominate.

INTRODUCTION

Brown adipose tissue (BAT) is now viewed as a major site of both non-shivering and diet-induced thermogenesis in small mammals (for reviews see Shrago & Strieleman, 1987; Himms-Hagen, 1989). The capacity for heat production in BAT is attributed to the loosely coupled respiration that can occur via the proton conductance pathway in mitochondria. Protons (or OH^-) can pass through the BAT mitochondrial inner membrane via uncoupling protein (UCP), thus bypassing ATP synthesis (for reviews see Nicholls & Locke, 1984; Nicholls *et al.* 1986). All BAT mitochondria contain UCP (also known as thermogenin), which is a tissue-specific protein (M_r 32000) that acts as a 'proton short-circuit', controlling the permeability of the inner membrane to protons. UCP is thus the principal factor regulating the uncoupling of respiration within the brown adipocyte (Nicholls & Locke, 1984).

UCP has a high affinity for the binding of purine nucleotides (Heaton *et al.*, 1978), and the binding of [^{3}H]GDP to BAT mitochondria is frequently used as an indicator of thermogenic activity. Whether GDP binding is in fact a direct measure of the UCP content of the mitochondria has been the subject of several conflicting reports. Some studies suggest that the ability of UCP to bind GDP can change rapidly in response to acute cold exposure, noradrenaline and pharmacological agents or dietary manipulations (Desautels *et al.*, 1978; Swick *et al.*, 1986; Swick & Swick, 1986; Milner *et al.*, 1988; Peachey *et al.*, 1988), and that the binding sites can therefore be masked and unmasked. Other reports, however, suggest that there is a fixed stoichiometry between the extent of GDP binding and the amount of UCP (Ricquier *et al.*, 1984; Nedergaard *et al.* 1984, 1989; Nedergaard & Cannon, 1985, 1987).

The majority of these studies have investigated the effects of environmental temperature changes on GDP binding and UCP concentration in order to learn how rapidly GDP-binding sites on BAT mitochondria can be unmasked and remasked. However, dietary signals have other important effects in BAT thermogenesis (Rothwell & Stock, 1979). The present paper studies the effects of different dietary signals on GDP binding and UCP concentration. We have investigated the existence of the unmasking/remasking process in adult rats made obese by feeding them on a cafeteria diet and in such obese rats on reversion to a standard diet. The effects of fasting have also been investigated. The changes associated with the overweight (obese) status *per se* and those associated with the cafeteria diet, the overfeeding of which results in obesity, were elucidated. The results indicate that the amount of GDP bound does not necessarily correlate with the UCP concentration, and that at least two mechanisms operate in the control of UCP activity, i.e. the unmasking/masking process and UCP turnover.

EXPERIMENTAL

Chemicals

[8-^{3}H]GDP and [U-^{14}C]sucrose were from Amersham International. Hydroxyapatite, Lubrol PX, reduced Triton X-100, Amberlite XAD-2, Freund's adjuvant, GDP, BSA (fraction V, essentially fatty-acid-free), rotenone, heparin, dithiothreitol (DTT), Tween-20, buffers, goat anti-rabbit IgG conjugated to

Abbreviations used: BAT, brown adipose tissue; UCP, uncoupling protein; DTT, dithiothreitol.

alkaline phosphatase, and *p*-nitrophenol phosphate were obtained from Sigma. Other chemicals were from Merck and Panreac.

Animals

Female Wistar rats were housed in wire mesh cages at 24 °C with a 12 h-light/12 h-dark lighting schedule. Tap water supplemented with minerals and vitamins and pelleted standard diet were provided *ad libitum* to all animals. Vitamins and minerals were added to water because the feeding of a cafeteria diet has been linked with vitamin deficiency (Moore, 1987), as the final nutrient composition of the diet is dependent on the animal's voluntary selection from the food items offered. Thus, in order to avoid any nutritional problems in this long-term study, we added a commercially available vitamin and mineral supplement (Vitachoc; Neosan Products, Barcelona, Spain) to the drinking water as previously described (Sclafani & Gorman, 1977; Prats *et al.*, 1986; Serra *et al.*, 1987; Roca *et al.*, 1989). After birth, rat pups were randomly assigned into eight dietary groups (ages given are those at the time of experiments): (1) 8-month-old control rats (C250), (2) 8-month-old cafeteria-diet-fed rats (O250), (3) 11-month-old control rats (C320), and (4) 11-month-old post-cafeteria-diet-fed rats (O320). The other four groups were the same but were fasted for 24 h before experiments (FC250, FO250, FC320, FO320). Until day 150, pelleted standard diet only was provided to all animals. The cafeteria diet plus the standard diet were offered from day 150 to day 250 of age, producing 8-month-old cafeteria-diet-fed rats. Animals in groups O320 and FO320 were fed on cafeteria diet plus standard diet from day 150 to day 250, and then on standard diet only from day 250 to day 320 (11 months old). The control groups always received standard diet. The rats were anaesthetized with pentobarbital (40 mg/kg) and killed by decapitation.

Diets

The quality and composition of the diets normally used in our laboratory have been previously described (Serra *et al.*, 1987). In brief, the cafeteria diet consisted of the following foods: cookies with foie-gras and sobrassada (a typical Majorcan sausage), sweets, bacon, biscuits, chocolate, peanuts, cheese, sugary milk and ensaïmada (a typical Majorcan cake). The standard diet (Panlab, Barcelona, Spain) was composed of (by wt.): 23.5% protein, 48.9% carbohydrate, 5.0% lipid, 12% water, 5.7% ash and 4.0% cellulose. The approximate composition of the cafeteria diet chosen by the rats was (by wt.): 13.6% lipid, 21.0% carbohydrate, 9.0% protein, 51.3% water and 5.1% others.

Determination of specific GDP binding to BAT mitochondria

BAT mitochondria from the interscapular site were prepared as described by Nedergaard & Cannon (1985), but the buffer used contained 10 mM-Hepes, 1 mM-EDTA, 1 mM-DTT, 0.25 M-sucrose and 0.005% heparin. The final mitochondrial pellet was resuspended in this buffer at a final concentration of 3 mg of protein/ml (Rodriguez-Vico *et al.*, 1989). GDP binding was measured as described previously (Sundin & Cannon, 1980) with slight modifications. Maximal GDP binding was measured in each experimental group. At the beginning of the experiment for each group we determined that 10 μM-GDP produced the maximum GDP binding assayed with 0.3 mg of mitochondrial protein, in accordance with Gribskov *et al.* (1986). Mitochondria (0.3 mg of protein) were incubated for 10 min at 30 °C in 2 ml of buffer containing 100 mM-[U-^{14}C]sucrose (6 kBq/ml), 10 μM-[8-^{3}H] GDP (ammonium salt) (10 kBq/ml), 20 mM-Tes, 1 mM-EDTA and 5 μM-rotenone. Non-specific binding was assessed by the addition of unlabelled GDP (25 μl of 10 mM-GDP). [U-^{14}C]-Sucrose was used to assay intermitochondrial space.

Purification of UCP

UCP was solubilized and purified from BAT mitochondria prepared from cold-adapted (3 weeks at 4 °C) 12-week-old Wistar rats. A modification of the method of Lin & Klingenberg (1980) was used. The mitochondrial pellet obtained from five rats as described above was incubated at 0 °C for 30 min in mitochondria isolation buffer (300 mM-sucrose, 2 mM-EDTA, 5 mM-2-mercaptoethanol and 10 mM-Tris/HCl, pH 7.2) containing 3.2% Lubrol PX. The membrane pellet was obtained by centrifugation for 1 h at 100000 *g*, and dissolved in 4 ml of a buffer containing 20 mM-Na_2SO_4, 1 mM-EDTA, 5 mM-2-mercaptoethanol and 20 mM-Mops (pH 6.7 with KOH). Reduced Triton X-100 was then added to a final concentration of 5%. The membranes were incubated at 0 °C for 30 min and immediately centrifuged as indicated above to obtain a supernatant containing the mitochondrial membrane proteins. The supernatant was warmed to room temperature and applied to a hydroxyapatite column containing 15 ml of hydroxyapatite and pre-equilibrated with 20 mM-Mops (pH 6.7) and 5 mM-2-mercaptoethanol. The column was eluted with this buffer at 30 °C with a flow rate of 2.5 ml/h. The dead volume was 12 ml, and a volume of 11 ml contained the maximum absorbance at 280 nm. This eluate was concentrated to 4 ml (speed vacuum) and then the protein was precipitated with acetone (1:5, v/v) at −20 °C overnight. The pellet obtained after 5 min of centrifugation at 16000 *g* was redissolved in 4 ml of the Mops buffer and applied to an Amberlite XAD-2 column (30 g of Amberlite XAD-2 pre-equilibrated with 10 mM-sodium phosphate buffer, pH 7.0 with KOH). The Amberlite column was eluted with this buffer at room temperature at a flow rate of 1.5 ml/min. After a dead volume of 14 ml, a peak at 280 nm with a volume of 8 ml appeared which corresponded to the UCP solution. This was finally stored at −20 °C at a concentration of 0.5 mg of protein/ml for subsequent analysis. These steps not only removed excess reduced Triton X-100 from the UCP solution but also improved the purity of UCP. The recovery of UCP from the total purification process was 5%. The purity of the protein was confirmed by SDS/PAGE (Hennessey & Scarborough, 1989) and immunoblotting as described below.

Antiserum preparation

An antibody to the purified UCP was raised in New Zealand White rabbits. The rabbits were immunized with an initial injection of 100 μg of purified UCP in Freund's complete adjuvant, and 28 days later a subsequent booster shot was given consisting of 60 μg of UCP in Freund's incomplete adjuvant. Rabbit blood was collected 1 week later (by heart puncture) and the serum fractions were isolated and stored frozen. The specificity of the antiserum to the UCP was confirmed by an immunoblot of BAT homogenate proteins, liver homogenate proteins and purified UCP as described below.

Immunoblotting

In order to confirm the specificity of the antiserum against rat UCP, an immunoblot was performed. The Interscapular BAT depot and liver was removed from a rat and homogenized in Tris/sucrose buffer (1.5 ml; 250 mM-sucrose/10 mM-Tris/HCl/1 mM-EDTA, pH 7.4) with a Teflon/glass homogenizer. The tissue homogenates were frozen at −20 °C prior to measurement of protein (Rodriguez-Vico *et al.*, 1989). For immunoblotting, the samples were denatured by addition of SDS/PAGE sample buffer (containing 62.5 mM-Tris base, 4% SDS, 5% 2-mercaptoethanol, 10% glycerol and 0.001% Bromophenol Blue, pH 6.8). Homogenates (20 μg of protein) of rat BAT and rat liver, and 4 μg of purified UCP were run on SDS/PAGE according to Laemmli (1970). Electrotransfer of separated proteins to

Hybond-C Extra nitrocellulose membranes (pore size 0.45 μm; Amersham) was carried out with an electroblotter (LKB) at 200 mA for 2 h according to Towbin *et al.* (1979). Blocking and development were performed according to Herron *et al.* (1990).

E.l.i.s.a. of UCP

A validation of the e.l.i.s.a. method has been carried out using different dilutions of antiserum and different amounts of recovered UCP in the well. The dilutions and the amounts of sample used gave absorbance values which could be read on the Multiscan photometer. First, 96-well microtitre plates (Nunc) were coated with 130 ng of UCP and incubated for 3 h at 37 °C, and then dried under hot air. The wells were then recoated with 100 μl of a 1% BSA solution in 0.9% NaCl/0.05% Tween and incubated for 3 h at 37 °C. The wells were then washed with 3×400 μl of NaCl/Tween.

Purified UCP (used as standard) and solubilized mitochondrial samples (0.125–1 μg of mitochondrial protein) were incubated with antibody (1:2000 final dilution) for 3 h at 37 °C. The standard curve was constructed over the range 0–100 ng. The contents of the wells were well mixed and the plate was incubated at room temperature for 2 h. The wells were then washed three times with NaCl/Tween.

For development, 100 μl of goat anti-rabbit IgG conjugated with alkaline phosphatase [diluted 1000-fold in phosphate-buffered saline (140 mM-NaCl/15 mM-NaH_2PO_4/Na_2HPO_4, pH 7.2) with 0.05% Tween 20] was added, and the plate was incubated overnight at 4 °C and then washed three times with NaCl/Tween. To each well was added 100 μl of *p*-nitrophenyl phosphate [in triethanolamine buffer (1 mg/ml; pH adjusted to 9.8 with HCl) containing 1 mM-$MgCl_2$], and the plate was incubated at room temperature. The development of absorbance at 405 nm was monitored with a Multiscan photometer and read after 60 min.

RESULTS

Under the conditions specified in the Experimental section, the reproducibility of GDP binding assays was within 5–8% of the S.D. and of the e.l.i.s.a. assays it was within 3–4% of the S.D.

The mean energy intake of the cafeteria-fed obese rats was 695 kJ/day, whereas in post-cafeteria and control rats it was 208 kJ/day. The excess weight gain was 60% in cafeteria rats (mainly related to hyperphagia) and 12% in post-cafeteria rats.

In the initial experiments the chronic effect of 100 days of cafeteria diet feeding on GDP binding and UCP concentration in 8-month-old rats was determined. In parallel with the increase in GDP binding, there was a chronic effect of cafeteria diet on the UCP concentration (Table 1). The binding ratio (nmol of GDP/nmol of UCP dimer) for control rats and treated rats was calculated to be the same in both groups, suggesting that GDP binding was increased because the UCP concentration was higher (76%) in cafeteria rats compared with control rats.

When the effects of 3 months of standard diet following 3 months of cafeteria diet on GDP binding and UCP concentration were studied (Table 1), there was a decrease in both parameters in standard-diet-fed obese rats compared with cafeteria-diet-fed obese rats, and the ratio between them was the same in both groups. Moreover, GDP binding and UCP concentration were higher (59%) in both cases than in controls (C320), although the ratio between them was not changed.

The effects of fasting on GDP binding and UCP concentration in controls, cafeteria-fed obese and standard-fed obese rats can be seen in Table 1. There were parallel decreases (55%) in GDP binding and UCP concentration in control rats, but, most importantly, in contrast with the large decrease in GDP binding in cafeteria- and standard-fed obese rats, there was no effect of fasting on the UCP concentration. The ratio of nmol of GDP bound/nmol of UCP in fasting obese groups was less than half that in the fasting control groups, suggesting that the activity of

Table 1. Effect of 3 months of cafeteria diet, 3 months of reversion to standard diet and 24 h of fasting on GDP binding and UCP concentration in interscapular rat BAT

Values represent means ± S.E.M. of 5–8 rats per group. C250, control rats at day 250 ($n = 5$); O250, cafeteria obese rats at day 250 ($n = 6$); FC250, fasted control rats at day 250 ($n = 5$); FO250, fasted cafeteria obese rats at day 250 ($n = 8$); C320, control rats at day 320 ($n = 5$); O320 post-cafeteria obese rats at day 320 ($n = 7$); FC320, fasted control rats at day 320 ($n = 5$), FO320 fasted post-cafeteria obese rats at day 320 ($n = 5$). Significant differences: * $P < 0.05$, ** $P < 0.01$ obese versus control, † $P < 0.05$, †† $P < 0.01$ fasted versus control, ‡ $P < 0.05$, ‡‡ $P < 0.01$ day 240 versus day 340; N.S., not significant. Individual differences from mean values were assessed by Student's *t* test. ANOVA gave *F* values for GDP binding, UCP and the molar binding ratio. The molar binding ratio was calculated per dimer of UCP.

Group	Treatment	GDP binding (nmol/mg of mitochondrial protein)	UCP concentration (μg/mg of mitochondrial protein)	Binding ratio (nmol of GDP/nmol of UCP)
C250		0.28 ± 0.04	71 ± 4	0.26 ± 0.04
O250		0.40 ± 0.04*	125 ± 15*	0.21 ± 0.02
FC250		0.15 ± 0.02†	31 ± 7†	0.31 ± 0.04
FO250		0.23 ± 0.04†	118 ± 14*	0.11 ± 0.01*†
ANOVA	Obesity	$P < 0.05$	$P < 0.01$	$P < 0.01$
	Fasting	$P < 0.01$	N.S.	N.S.
	Interaction	N.S.	N.S.	$P < 0.05$
C320		0.14 ± 0.01‡	34 ± 4‡‡	0.26 ± 0.02
O320		0.212 ± 0.02*‡‡	53 ± 4‡‡	0.28 ± 0.03
FC320		0.11 ± 0.02	23 ± 3	0.28 ± 0.04
FO320		0.12 ± 0.01††‡	60 ± 4**‡	0.128 ± 0.01*†
ANOVA	Obesity	$P < 0.05$	$P < 0.01$	$P < 0.05$
	Fasting	$P < 0.01$	N.S.	$P < 0.05$
	Interaction	N.S.	$P < 0.05$	$P < 0.05$
Age		$P < 0.01$	$P < 0.01$	N.S.

UCP (GDP binding) was decreased not by a specific loss of UCP but by a decrease in the available GDP-binding sites. There was no difference in GDP binding when comparing the fasted control groups with the fasted obese groups. However, the obese groups maintained higher UCP concentrations than the controls, thus explaining the lower ratio in the former animals.

DISCUSSION

GDP-binding studies have been widely used as a method of assessing the thermogenic state of BAT mitochondria *in vitro*, but whether the binding studies are a measure of the concentration of UCP is not yet clear (Himms-Hagen, 1989). There is evidence to suggest that the extent of GDP binding can be dissociated from the UCP content of BAT mitochondria (Desautels *et al.*, 1978; Gribskov *et al.*, 1986; Trayhurn *et al.*, 1987; Milner *et al.*, 1988; Swick & Henningfield, 1989), i.e. rapid changes in GDP binding have been measured without any apparent changes in the amount of UCP. Other studies, however, have failed to detect any rapid change in the ability of UCP to bind GDP, and a fixed stoichiometry for the binding of the ligand to UCP has been postulated (Holloway *et al.*, 1984; Nedergaard *et al.*, 1984: Rial & Nicholls, 1984; Ricquier *et al.*, 1984; Nedergaard & Cannon, 1985). In this case, the extent of GDP binding would represent a direct measure of UCP concentration.

Feeding rats on a cafeteria diet for 3 months induced increases in both GDP binding and UCP concentration. This result is in agreement with the studies of Rothwell & Stock (1983), which investigated the effect of 6.5 months of cafeteria diet feeding of rats on GDP binding (GDP binding was increased but this did not attain statistical significance). It is also in agreement with the parallel increases observed in GDP binding and UCP concentration in 10-week-old cafeteria-fed rats (Nedergaard *et al.*, 1984). Another dietary manipulation, i.e. feeding a 5% lactalbumin diet to rats for 3 weeks (compared with a 15% lactalbumin diet) resulted in stimulated BAT activity as measured by GDP binding, but did not alter the mean concentration of UCP (Swick & Swick, 1986). These results indicate that the nutritional status of the animal can change the activity and/or concentration of UCP that is associated with the thermogenic response.

Reversion to a standard diet for 3 months after 3 months of cafeteria feeding apparently resulted in a decrease in both parameters (GDP binding and UCP concentration). However, 11-month-old control rats also exhibited decreased values of GDP binding and UCP concentration compared with 8-month-old control and rats. Thus the changes are better related to age. They could be due to the involution of BAT which occurs in older animals (Rothwell & Stock, 1983).

The UCP-related thermogenic response to fasting was different in the different groups studied. In control rats there was a selective loss of UCP. Previous results in mice were controversial, with Trayhurn & Jennings (1986) obtaining results similar to ours, whereas Desautels (1985) found no changes in the amount of UCP. In hamsters, fasting does not induce decreases in GDP-binding or UCP concentration, but there is a loss of mitochondrial mass (Levin & Trayhurn, 1987). Fasting induced a decrease in GDP binding of the same order as the decrease in amount of UCP; thus the value of GDP/nmol of UCP was maintained at 0.25. UCP is considered to exist as a dimer, each dimer having one binding site for GDP (Lin & Klingenberg, 1990). The binding ratio (nmol of GDP/nmol of dimer) in the present study was practically the same for both control and fasted rats, at a value suggesting that one-quarter of the theoretical number of binding sites were occupied. In both cafeteria and post-cafeteria obese rats there was a fasting-induced masking of UCP, i.e. starvation produced a decrease in GDP binding without any loss of UCP. The different mechanisms shown here for control and obese rats can be tentatively related to their different hormone/fuel metabolic responses to food deprivation, the obese rats using mainly lipid and conserving α-amino nitrogen, compared with controls (Gianotti *et al.*, 1988; Roca *et al.*, 1989). The response to fasting in post-cafeteria obese rats was very similar to that in cafeteria obese rats, with a decrease in BAT activity during the fast caused by a decrease in GDP binding, without any decrease in UCP concentration. Therefore the inactivation of the mitochondrial conductance pathway probably occurs by the masking of UCP to an inactive form, thus avoiding passage of H^+ (or OH^-) across the mitochondrial inner membranes.

In previous studies (Desautels *et al.*, 1986; Trayhurn *et al.*, 1987) it was shown that UCP, when it is first incorporated into the mitochondria, is fairly stable and has a half-life of the order of 1–2 weeks. However, in a deacclimatization situation, where the total amount of mitochondria is also decreasing, the loss of total UCP is markedly quicker, and total UCP half-life then seems to be only a few days. In our fasted control rats the UCP concentration decreases by 40 μg/mg of mitochondrial protein in 24 h, a value that implies a UCP half-life of about 13 h. This half-life was increased in fasted controls until it attained a value of nearly 34 h on day 300. These values of the apparent half-life have been calculated from the data of Table 1. In the fasted obese rats we have not been able to calculate the half-life of UCP because there was not any change in the amount of the protein. This result could suggest that the UCP half-life was not decreased in these groups, thus maintaining values similar to those in fed rats.

Our results provide no insight into the mechanism of the masking process, but show differences in UCP parameters between obese and control rats. Changes in lipid and protein metabolism during the fast can affect masking of UCP, and its turnover is unknown. Non-esterified fatty acids, which show an increased availability in obese rat tissues, are activators of UCP during fasting, when this protein is masked. Conformational changes in the protein within the membrane, monomerization of pre-existing dimers, translocation and removal of UCP out of the inner membrane to a store within the mitochondrial matrix, and covalent modifications, are possibilities previously suggested (Peachey *et al.*, 1988). Nedergaard & Cannon (1987) suggested that unmasking of GDP-binding sites could result from mitochondrial swelling, as indicated by changes in the volume of the mitochondrial matrix. However, Swick & Swick (1986) were unable to demonstrate any changes in GDP binding associated with mitochondrial swelling. Irrespective of the precise meaning of masking of UCP, the present study demonstrates a different response of the main UCP-related thermogenic parameters in control rats and cafeteria-fed obese rats, and that this difference is related to the obese status *per se* rather than to the composition of the cafeteria diet. Thus, during the fast, the values of the ratio (nmol of GDP/nmol of UCP) in the two obese groups were similar, but lower than that in controls, suggesting a response independent of diet.

The present study is consistent with the view that binding sites for GDP on UCP can be masked (Henningfield & Swick, 1987; Milner *et al.*, 1988). The results also indicate that changes in GDP binding during 24 h of fasting in cafeteria and post-cafeteria obese rats appear to be related to inactivation of UCP, without its loss, whereas in control rats 24 h of fasting results in a selective loss of UCP. GDP binding, rather than of the amount of UCP, appears to be a measure of thermogenic activity, although over the long term the two parameters show parallel

changes (see Trayhurn *et al.*, 1987; Milner *et al.*, 1988). Our results indicate that masking/unmasking of UCP could reflect a response of the mitochondrial proton conductance pathway in BAT to physiological stimuli, such as environmental temperature or dietary signals. There are at least two mechanisms that could be involved in the regulation of UCP: (1) the control of its activity as measured by GDP binding, in that masking/unmasking appears to result in inactive/active states of UCP; and (2) control of UCP turnover.

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