Physiology & Behavior 104 (2011) 893–899

Contents lists available at ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/phb

Leanness of Lou/C rats does not require higher thermogenic capacity of brown adipose tissue

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ARTICLE INFO

Article history:
Received 12 July 2010
Received in revised form 20 May 2011
Accepted 24 May 2011

Keywords:
Free running-wheel
Lou/C rats
Obesity
Cold exposure
Brown adipose tissue
Thermogenesis
UCP1

ABSTRACT

Lou/C rats, an inbred strain of Wistar origin, remain lean throughout life and therefore represent a remarkable model of obesity resistance. To date, the exact mechanisms responsible for the leanness of Lou/C rats remain unknown. The aim of the present study was to investigate whether the leanness of Lou/C rats relies on increased thermogenic capacities in brown adipose tissue (BAT).

Results showed that although daily energy expenditure was higher in Lou/C than in Wistar rats, BAT thermogenic capacity was not enhanced in Lou/C rats kept at thermoneutrality as demonstrated by reduced thermogenic response to norepinephrine *in vivo*, similar oxidative activity of BAT isolated mitochondria *in vitro*, similar levels of UCP1 mRNA and lower abundance of UCP1 protein in interscapular BAT depots. Relative abundance of β_3-adrenergic receptor mRNA was lower in Lou/C BAT while that of GLUT4, FABP or CPT1 was not altered. Activity-related energy expenditure was however considerably increased at thermoneutrality as Lou/C rats demonstrated an impressively high spontaneous running activity in voluntary running wheels. Prolonged cold-exposure (4 °C) depressed the spontaneous running activity of Lou/C rats while BAT thermogenic capacity was increased as reflected by rises in BAT mass, oxidative activity and UCP1 expression. It is concluded that the leanness of Lou/C rats cannot be ascribed to higher thermogenic capacity of brown fat but rather to, at least in part, increased locomotor activity. BAT is not deficient in this rat strain as it can be stimulated by cold exposure when locomotor activity is reduced suggesting some substitution between these thermogenic processes.

1. Introduction

The prevalence of obesity in humans continues to rise in industrialized countries, mainly in children, and constitutes a pressing problem of public health. Although multifactorial, the development of obesity results from an imbalance between caloric intake and energy expenditure that leads to body fat accumulation. Excessive body fat is associated with increased risk of chronic metabolic diseases such as atherosclerosis and diabetes [1,2]. Most of the genetic studies have focused on genes and polymorphisms associated with the obese phenotype. Considerably less attention has been paid to understand why certain people remain thin and do not develop obesity under the same environmental pressure (sedentary life, fat and energy rich foods). Many genetic manipulations and cross-breeding have created animal models of obesity providing an insight into the molecular mechanisms that affect energy balance and contribute to fat accretion. In contrast, few animal models are available to understand the molecular basis of leanness and resistance to obesity.

A valuable model of obesity resistance is the Lou/C rat [3], an inbred strain of Wistar origin that does not develop obesity with high fat diet [4] and age [5] contrary to Wistar rats. Accordingly, Lou/C rats exhibit reduced body weight at all ages as compared to Wistar rats and percentage of fat is relatively stable throughout life [6]. Although the exact mechanisms responsible for the leanness of Lou/C rats remain unknown. It may imply i) a reduced energy intake, and/or ii) an enhanced energy expenditure. To date, it has been shown that Lou/C rats presented no reduction in food intake compared with Wistar or Fischer F344 rats [4,7–9], but exhibited higher daily energy expenditure [8,9]. Such increased energy expenditure may contribute to burn out food energy and thus reduce its storage in white adipose tissue. In rodents, brown adipose tissue (BAT) contributes to a significant part of energy expenditure on account of its large oxidative capacities [10]. It has thus been proposed that a defect in BAT activity may be involved in the development of obesity [11]. Indeed, BAT-associated facultative thermogenesis is altered in obese rodents in connection with a reduction of uncoupling protein 1 (UCP1) expression [11]. Conversely, it may be postulated that the leanness

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doi:10.1016/j.physbeh.2011.05.029

of Lou/C rats may rely on hyperactive BAT dissipating as heat excessive food energy intake. Accordingly, Abdoulaye and co-workers showed that the respiratory rate and proton conductance of mitochondria extracted from Lou/C BAT were higher than in Fischer 344 rats [7]. However, it cannot be excluded that such effect might also result from a very low thermogenic activity of mitochondria from the obesity-prone Fischer 344 rats.

The aim of the present study was to investigate the potential increased thermogenic capacity of BAT in Lou/C rats as compared with age-matched Wistar rats, the rat strain from which Lou/C rats derive. Understanding the determinants of the outstanding leanness of Lou/C rats may yield important and complementary findings to the study of obesity models.

2. Methods

2.1. Animals

Our study was performed in accordance with the recommendation provided by the European Convention for the protection of Vertebrate Animals used for Experimental and Scientific purposes (Council of Europe n° 123, Strasbourg, 1985).

Two inbred strains of rats were used: male Lou/C rats ($n=23$; Harlan, France) and male Wistar rats ($n=23$; Harlan, France). At the age of 12 weeks, animals were housed six per box under a 12 h light/dark cycle, and allowed to acclimatise to their new conditions for 1 week before the study. Then, rats were housed individually and maintained in a temperature-controlled room (25 ± 1 °C) for three weeks before the beginning of experiments. After this period, the rats were split into different experimental groups described in "Procedure" section. Food (A04 – Scientific Animal Food & Engineering, France) and water were available *ad libitum*. Rat body mass was measured weekly until 22 weeks of age. Food consumption was estimated twice a week during all the experiments as the difference between the amount of food given and that removed from the cage. Spilling was taken into account as much as possible. Results were expressed per kg of body mass or per kg of fat free mass determined after post-mortem dissection of carcasses.

2.2. Procedure

2.2.1. Spontaneous locomotor activity

Wistar and Lou/C rats ($n=6$) were placed for three weeks at 25 °C in cages set up with a free-access exercise wheel (Bionox, Ancy, France) attached to one side of the cage. Food intake and body weight were determined once a week while spontaneous activity was monitored continuously.

2.2.2. Brown adipose tissue capacity

Recruitment of thermogenic capacity in BAT was investigated classically *in vivo* by the calorigenic response to a test injection of norepinephrine (NE, Sigma) [12]. Wistar and Lou/C rats ($n=5$ per group), 20–22 weeks old, were placed at thermoneutrality and their basal energy expenditure was monitored by indirect calorimetry. We used an open-circuit respirometer and gas analysing system as previously described [13]. Rats were positioned in a thermostatic chamber ventilated by a constant atmospheric airflow (4 L/min). Variable heat loss by conduction on the ground was minimized by a polypropylene bed. Ambient temperature (Ta) were controlled and measured with copper-constantan thermocouples inside the thermostatic chamber. Air flow rates were measured using a Platon volumeter, and converted to standard values (STPD). The fractional concentrations of oxygen were measured using a Servomex 1100 paramagnetic gas analyzer (Taylor Instrument Analytics ltd, Sussex, UK). Carbon dioxide concentrations were measured using a Servomex 1400 infrared gas analyzer. Analysers were calibrated daily and the rates of O_2 consumption and CO_2 production as well as the caloric equivalent for O_2 determined from the respiratory quotient were calculated as described previously [13].

The thermogenic response to norepinephrine was measured after a single intraperitoneal injection of norepinephrine (0.3 mg/kg) as compared with saline. Energy expenditure (W/kg) was monitored before (resting) and after the injection. Resting energy expenditure represents the lower level measured for at least 10–15 min periods between bursts of activity easily detected by peaks of O_2 uptake and CO_2 production on records. The thermogenic response to NE was calculated as the net increase in metabolic rate (maximal energy expenditure measured after injection minus basal energy expenditure).

Brown fat thermogenic activation was then estimated by the functional activity of isolated mitochondria and the abundance of UCP1. Batches of Wistar or Lou/C rats ($n=6$ per group) were reared at either thermoneutrality 25 °C or 4 °C for three week. Such cold-acclimation protocol is known to induce metabolic activation of BAT in rodents [10]. Animals were killed by decapitation. Interscapular BAT (iBAT) and visceral fat pad (retroperitoneal and epididymal white adipose tissue) were rapidly excised and weighed. The viscera, including the liver, lungs, heart, and the gastrointestinal tract were removed. The remainder of the body, henceforth called the carcass, was digested in hot 30% KOH. The lipid content of this mixture was assumed to be representative of peripheral fat [14]. Taking account for visceral and peripheral fat, we obtained body composition of Wistar and Lou/C rats. A small portion of iBAT was immediately frozen in liquid nitrogen and stored at −80 °C for reverse transcription polymerase chain reaction (RT-PCR) while the remaining was used for isolation of mitochondria by differential centrifugation as described previously [15]. Mitochondrial protein content was determined by the bicinchoninic acid (BCA) method (Pierce, Rockford, IL). Mitochondrial oxygen consumption was determined by polarography (Rank Brothers Ltd) at 37 °C in a medium containing 125 mM KCl, 1 mM EGTA, 2 mM KH_2PO_4, 20 mM Tris–HCl with 0.3% free fatty-acid BSA (pH 7.2). The control state of respiration (state 2) was initiated by the addition of 5 mM succinate, in the presence of 2 μM rotenone. To demonstrate the contribution of UCP1 to mitochondrial respiration, 1 mM of guanosine diphosphate (GDP), a known inhibitor of UCP1, was added to the mitochondrial suspension. Remainder of mitochondria was stored at −80 °C for western blot analysis.

The relative abundance of target mRNA was determined by semi-quantitative RT-PCR using β-actin as standard. RT was performed in a thermocycler Thermo Hybaid (Ashforf, UK) using 1 μg of total RNA extracted with Tri Reagent (Euromedex, France) Amplified fragments were separated on gels and their relative band intensities (ratio of each target to β-actin) were determined by scanning densitometry with a Kodak Digital Science 1D 2.0 software (Kodak Scientific Imaging System). Primers (sens and antisens) were GTG AAG GTC AGA ATG CAA GC and AGG GCC CCC TTC ATG AGG TC for UCP1 (gi6981691), GAGGCAACCTGCTGGTAATCAC and GAG TGA CAC TCT TGC GCC TCA G for β3 adrenergic receptor (gi6978462), CAG ATC GGC TCT GAA GGT GC and CTG AGT AGG CGC CAA TGA G for GLUT4 (glucose transporter 4, gi464195), CTG GAA GCT AGT GGA CAG and GAC TTG ACC TTC CTG TCA TC for FABP (fatty-acid binding protein, gi204079), TAT GTG AGG ATG CTG CTT CC and CTC GGA GAG CTA AGC TTG TC for CPT1 (carnitine palmitoyl transferase 1, NM_031559).

The relative abundance of UCP1 was determined at the protein level. The remaining frozen iBAT mitochondria were used in western blot analysis. Briefly, 20 μg of iBAT mitochondria proteins were separated by SDS-PAGE (12.8% acrylamide) and transferred to polyvinyldene fluoride membranes (Immobilon-P, Millipore). Immunological detection was performed using a rabbit antiserum against UCP1 (α-diagnostics UCP11-A, USA). The detection was realized with a horseradish peroxidase-coupled anti-rabbit (Bio-Rad 170–6516) secondary antibody and an enhanced chemiluminescence (ECL)