Experimental and Toxicologic Pathology 65 (2013) 549–557

Contents lists available at SciVerse ScienceDirect

Experimental and Toxicologic Pathology

journal homepage: www.elsevier.de/etp

Responses of brown adipose tissue to diet-induced obesity, exercise, dietary restriction and ephedrine treatment

Nikki Slocum[a], Jessica R. Durrant[b], David Bailey[a], Lawrence Yoon[a], Holly Jordan[a], Joanna Barton[a], Roger H. Brown[a], Lisa Clifton[c], Tula Milliken[c], Wallace Harrington[c], Carie Kimbrough[d], Catherine A. Faber[e], Neal Cariello[a], Chandikumar S. Elangbam[a,*]

[a] *Safety Assessment, GlaxoSmithKline, Research Triangle Park, NC, USA*
[b] *College of Veterinary Medicine, North Carolina State University, Raleigh, NC, USA*
[c] *Muscle Metabolism Discovery Performance Unit, GlaxoSmithKline, Research Triangle Park, NC, USA*
[d] *Statistical Sciences, GlaxoSmithKline, Research Triangle Park, NC, USA*
[e] *College of Veterinary Medicine, Purdue University, IN, USA*

ARTICLE INFO

Article history:
Received 7 February 2012
Accepted 3 April 2012

Keywords:
Brown adipose tissue
Thermogenesis
Obesity
Gene expression
Diet-induced obese mice

ABSTRACT

Drug-induced weight loss in humans has been associated with undesirable side effects not present in weight loss from lifestyle interventions (caloric restriction or exercise). To investigate the mechanistic differences of weight loss by drug-induced and lifestyle interventions, we examined the gene expression (mRNA) in brown adipose tissue (BAT) and conducted histopathologic assessments in diet-induced obese (DIO) mice given ephedrine (18 mg/kg/day orally), treadmill exercise (10 m/min, 1-h/day), and dietary restriction (DR: 26% dietary restriction) for 7 days. Exercise and DR mice lost more body weight than controls and both ephedrine and exercise reduced percent body fat. All treatments reduced BAT and liver lipid accumulation (i.e., cytoplasmic lipids in brown adipocytes and hepatocytes) and increased oxygen consumption (VO2 ml/kg/h) compared with controls. Mitochondrial biogenesis/function-related genes (TFAM, NRF1 and GABPA) were up-regulated in the BAT of all groups. UCP-1 was up-regulated in exercise and ephedrine groups, whereas MFSD2A was up-regulated in ephedrine and DR groups. PGC-1α up-regulation was observed in exercise and DR groups but not in ephedrine group. In all experimental groups, except for ephedrine, fatty acid transport and metabolism genes were up-regulated, but the magnitude of change was higher in the DR group. PRKAA1 was up-regulated in all groups but not significantly in the ephedrine group. ADRß3 was slightly up-regulated in the DR group only, whereas ESRRA remained unchanged in all groups. Although our data suggest a common pathway of BAT activation elicited by ephedrine treatment, exercise or DR, mRNA changes were indicative of additional nutrient-sensing pathways in exercise and DR.

1. Introduction

Excess energy intake over expenditure results in obesity, and it has been estimated that more than 50% of the European population are overweight (Body Mass Index, BMI > 25 kg/m^2) and 30% are obese (BMI > 30 kg/m^2) (Groves, 2006; Information Centre, 2008). In the United States, adult obesity and overweight (combined) prevalence was 68.0% of the population during 2007–2008 (Flegal et al., 2010). Furthermore, complications related to obesity (e.g., cardiovascular disease, type 2 diabetes, hypertension, metabolic syndrome, nonalcoholic fatty liver disease and cancer) continue to burden the health care system (an estimated expense of $147 billion a year) in the United States (Finkelstein et al., 2009; Booth et al., 2011). Common strategies to obtain a healthy BMI include changes in diet and exercise. But these lifestyle changes are not easily achieved, and most importantly, results may be moderate and transient. Recently, brown adipose tissue (BAT) has been proposed as a target to modify energy expenditure. BAT has been shown to maintain core temperature via thermogenesis by dissipating stored energy as heat. Morphologically, brown adipocytes have granular eosinophilic cytoplasm due to high content of large mitochondria with abundant cristae. These adipocytes are densely innervated by the sympathetic nervous system, and sympathetic neuronal release of norepinephrine activates ß-adrenergic receptors (ßARs) expressed on the surface of brown adipocytes, ultimately leading to the production of heat. Heat is generated by uncoupling oxidative

* Corresponding author at: Safety Assessment, 9.3010.2D, GlaxoSmithKline, 5 Moore Drive, Research Triangle Park, NC 27709-3398, USA. Tel.: +1 919 483 0043; fax: +1 919 483 0692.
E-mail address: chandi.s.elangbam@gsk.com (C.S. Elangbam).

0940-2993/$ – see front matter © 2012 Elsevier GmbH. All rights reserved.
http://dx.doi.org/10.1016/j.etp.2012.04.001

phosphorylation from ATP production via uncoupling protein-1 (UCP-1), a BAT-specific proton transporter located in the inner mitochondrial membrane. In rodents, UCP-1 has been shown to play an important role in the regulation of energy expenditure and body weight. BAT activation causes body weight loss in rodents, and is likely to be protective against obesity-associated diseases (Ghorbani et al., 1997; Guerra et al., 1998).

In the past, BAT was thought to exist only in small mammals and infants, but recent data from positron emission tomography and computerized tomography have confirmed the presence of BAT depots in adult humans (Virtanen et al., 2009; Cypess and Kahn, 2010). It is now clear that human BAT depots are activated by cold exposure and adrenergic drugs (Cypess and Kahn, 2010). Mounting evidence suggests that BAT is protective against obesity and weight gain in humans. It has been estimated that as little as 50 g of maximally stimulated human BAT could utilize up to 20% of basal caloric needs (Rothwell and Stock, 1983), and therefore, the ability to stimulate energy expenditure via BAT thermogenesis might have important implications for the treatment of obesity and concurrent diseases in humans. Targeting BAT thermogenesis by drugs is a novel anti-obesity approach; however, it is critical to compare this drug-induced weight loss strategy with weight loss approaches that are generally considered safe, such as diet and exercise. In this study, we examined BAT adaptation to exercise, diet restriction (DR) and ephedrine, a sympathomimetic drug, in diet-induced obese (DIO) mice. Our aim was to better elucidate efficacy and toxicity-driven mechanism of weight loss and to characterize the mechanistic differences among these weight loss strategies by evaluating changes in gene expression (mRNA), body composition, CLAMs (Comprehensive Laboratory Animal Monitoring system) and histopathology in DIO mice.

2. Materials and methods

All procedures involving animals conformed to the guidelines set forth in the Guide to the Use and Care of Laboratory Animals and were reviewed and approved by the GlaxoSmithKline Institutional Animal Care and Use Committee. 22–24 Weeks old, male DIO (C57Bl/6NTac) mice were obtained from Taconic (Germantown, NY). Except for the DR group, all animals were fed a standard obesity inducing high fat diet (60% kcal from fat; Research Diets, Inc., New Brunswick, NJ) ad libitum. Reverse osmosis-treated water was available ad libitum and mice were housed in standard polycarbonate caging. Treatment groups were randomized by body weight and fat/lean composition after initial measurements were recorded. Body weight, food and water intake were recorded daily. Clinical observations were performed prior to and 2-h post treatment. All animals were dosed via oral gavage at 10 ml/kg daily for seven days in the A.M, with either vehicle (sterile water, 10 ml/kg) or ephedrine (Sigma chemical; (1R,2S)-(−)-Ephedrine, Purity: 99%, Lot number: S44211-339, Expiration Date: 13 September 2012) in sterile water. To avoid immediate toxic drug effects, the ephedrine dose group ($n=6$) was treated at 12.5 mg/kg/day for the first 3 days and then raised to 18 mg/kg/day for the last 4 days. Control mice ($n=5$) were sedentary and gavaged daily with vehicle (sterile water). The DR group ($n=5$) was subjected to 26% dietary restriction. Mice in the exercise group ($n=5$) were gavaged daily with vehicle (sterile water) prior to exercise. The mice ran 1 h each morning on an Eco 3/6 mouse treadmill (Columbus Instruments, Columbus, OH) for 7 days at 10 m/min, 0% grade. When mice failed to maintain the pace, they were lightly touched with a brush (which stimulates avoidance running) or received a mild shock from the grid at the end of the treadmill. Most mice ran voluntarily the entire hour.

2.1. In-life measurements

Quantitative nuclear magnetic resonance imaging (qNMR) was utilized to assess body composition before and after treatments. Oxygen consumption, carbon dioxide release and body temperature were recorded over a 24 h period using the Comprehensive Lab Animal Monitoring System (CLAMS) cage (Columbus Instruments, Columbus, OH), prior to and after the seven-day treatment period. CLAMS continuously and simultaneously monitors food and water intake, indirect calorimetry, and *x*- and *z*-axis activity to monitor movement. Animals had unrestricted access to powdered chow through a feeder located in the middle of the cage floor. Animals were acclimated to the CLAMS prior to testing.

2.2. Necropsy

Twenty-four hours after the last ephedrine and vehicle treatments, animals were euthanized via exsanguination from the abdominal vena cava while under isofluorane anesthesia. Prior to euthanasia, a non-fasted serum sample was collected from the abdominal vena cava for analysis of select clinical chemistry endpoints. The following serum endpoints were analyzed on an automated clinical chemistry analyzer (Olympus AU640e, Beckman Coulter, Inc., Brea, California) by routine methods: glucose, total cholesterol, triglycerides, glycerol, beta-hydroxybutyric acid, and non-esterified fatty acids. Complete necropsies were performed on all animals after exsanguination. The interscapular BAT was removed and a portion was snap frozen in liquid nitrogen for TaqMan™ (Real-time RT-PCR). The remaining BAT and other tissues were preserved in 10% neutral buffered formalin, processed, sectioned and stained with hematoxylin and eosin for histopathological evaluation.

2.3. RNA isolation and TaqMan (Real-time RT-PCR)

BAT snap frozen in liquid nitrogen was homogenized on using a rotor homogenizer in 4 ml of Trizol. A 700 μl aliquot of lysate was transferred to a PLG (phase lock gel) tube and 140 μl of chloroform was added and shaken. Lysate was spun at 16 000 × *g* for 4 min. 300 μl of aqueous supernatant was transferred to a 1.5 ml tube and 450 μl of 100% ethanol was added and mixed. Supernatant and ethanol mix was transferred to an RNeasy Mini column (Qiagen). The column was washed with 700 μl of RW1, and two following washes of 500 μl of RPE buffer. The column was spun at 16 000 × *g* for 3 min to dry. 30 μl of dH_2O was used to elute RNA from the column. RNA samples were then DNased-treated using Turbo DNAfree (Ambion). 1 μl of sample was used to measure concentration on a Nanodrop (Thermo), and used to calculate volume for 1 μg of RNA. The 1 μg of RNA was then used to reverse-transcribe RNA into cDNA using Applied Biosystems' High Capacity RNA to cDNA Master Mix kit. cDNA was then diluted 1:50 with dH_2O and used as template for Taqman. 5 μl of diluted cDNA was added to 15 μl of mastermix + primers on a 384 well plate. Samples were run using an Applied Biosystems' 7900HT. Primers were purchased from Applied Biosystems gene expression assay catalog.

2.4. Statistical analysis

Statistical analysis was performed using SAS, Version 9.2 (SAS Institute Inc., Cary, NC). Difference in body weight change, average food and water consumption, average KCal, clinical chemistry and qNMR data was analyzed using *t*-tests via contrast statements in SAS Proc MIXED (MIXED Procedure of SAS/STAT). Due to the number of comparisons performed, the *p*-values were adjusted via a Bonferroni–Holm multiple comparison adjustment using SAS Proc