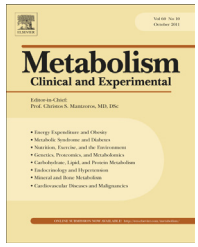
Available online at www.sciencedirect.com

Metabolism

www.metabolismjournal.com



Clinical Science

Irisin levels correlate with energy expenditure in a subgroup of humans with energy expenditure greater than predicted by fat free mass

Andrew G. Swick, Stephen Orena, Annalouise O'Connor*

UNC Nutrition Research Institute, University of North Carolina Chapel Hill, North Carolina Research Campus, Kannapolis, North Carolina, USA

ARTICLE INFO

Article history:
Received 7 December 2012
Accepted 27 February 2013

Keywords:
FNDC5
BAT
Indirect calorimetry
RQ

ABSTRACT

Objective. Obesity is a result of chronic overconsumption of calories relative to the amount of energy expended. While fat free mass can account for ~80% of the variance in energy expenditure, there is still considerable variability in energy requirements between individuals that cannot be explained. We hypothesized that responsiveness to the recently discovered myokine, irisin, which has been touted to increase energy expenditure via activation of brown adipocytes in rodents and possibly humans, may explain some of the variability in energy expenditure.

Materials/methods. Post-menopausal women (n = 17) spent 24-h in a whole room indirect calorimeter. During the study day, subjects remained sedentary and consumed meals tailored to their energy requirements. Plasma irisin, leptin and adiponectin were measured in samples taken from each subject.

Results. Our results suggest that in general, irisin levels do not correlate with 24-h energy expenditure, however, for a subpopulation irisin levels and energy expenditure are highly correlative.

Conclusion. Irisin may help explain some of the observed variability in individual energy requirements that cannot be accounted for by fat free mass. Therefore, interventions designed to increase irisin action may prove to be promising avenues for the treatment of obesity.

1. Introduction

Obesity is an ever-increasing, worldwide health concern associated with a significant economic burden. Obesity results from chronic overconsumption of calories (kcal) relative to the amount of calories expended. While fat free mass (FFM) can explain approximately 80% of the variance in 24 h energy expenditure (EE) [1], there is still considerable variability in energy requirements between individuals. In adults, EE is generally correlated with FFM, but there is significant variability between individuals with similar FFM, that cannot be explained by age, BMI, sex or any other phenotypic characteristic. The 20% variation in EE has a potentially tremendous impact on the prevalence and severity of obesity in the general population. In spite of this, understanding the underlying biology of this difference, identification of novel genes

Abbreviations: BAT, brown adipose tissue; BMI, body mass index; DXA, dual x-ray absorptiometry; EE, energy expenditure; FFM, fat free mass; FM, fat mass; FNDC5, fibronectin type III domain containing 5; PGC-1α, peroxisome proliferator-activated receptor γ coactivator-1α; RQ, respiratory quotient; VO_2, volume oxygen consumed (liters/min); VCO_2, volume CO_2 expired (liters/min).

Clinical Trials Registration Number: NCT01729143.

* *Corresponding author.* Tel.: +1 704 250 5015; fax: +1 704 250 5001.
E-mail address: andrew_swick@unc.edu (A.G. Swick).

0026-0495/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.metabol.2013.02.012

regulating EE and development of effective therapies to modulate EE have been elusive.

One potential biological source of variable EE is brown adipose tissue (BAT) which contributes to EE in some animals, including small rodents such as mice and rats. Although BAT is present in newborn humans, its existence and function in adult humans have been debated [2]. Recent reports using positron emission tomography suggest the presence of active BAT in some, but not all adults [3,4]. Furthermore, there appears to be a correlation between the presence of active BAT and increased EE in response to stimuli such as cold exposure and consumption of capsaicin [5,6]. The regulation of BAT in humans is poorly understood, however Bostrom et al. recently reported the discovery of irisin, a myokine that stimulates the development of BAT and beige adipose tissue in mice and in cell culture [7,8]. Levels of circulating irisin increase in response to chronic exercise in mice [9] and humans [10] and administration of irisin to mice results in increased EE [9]. Furthermore, irisin levels are reported to be decreased in patients with type 2 diabetes, while positively correlated with BMI, fat mass and muscle mass across a very broad spectrum of body weight [11,12] We hypothesized that irisin action could contribute to and account for the differential in EE in individuals whose EE is greater than predicted by FFM, since the BAT component of EE is not accounted for by calculations using FFM.

2. Methods and procedures

2.1. Study subjects

The study subjects were post-menopausal women with BMI between 24 and 45. Other inclusion criteria included age between 50 and 70 years; no illness or medical condition that may affect the results (e.g. diabetes); does not exercise heavily (defined as > 150 min/week for greater than 3 months).

2.2. Fat free mass and predicted energy expenditure

Body composition (fat mass and fat free mass (FFM)) was determined via dual energy x-ray absorptiometry (DXA) (GE Lunar iDXA; Milwaukee, WI). Resting Metabolic Rate (RMR) was calculated using an FFM-based equation [418 + (20.3*FFM)] [13]. Predicted 24 h EE was calculated as RMRX 1.3 (physical activity level (PAL)) whilst in the metabolic chamber.

2.3. Indirect calorimetry

Indirect calorimetry was conducted using the metabolic chamber located at the UNC Nutrition Research Institute, Kannapolis, NC. The chamber was modelled on chambers at the National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD [14], and is an open-circuit, pull-type, whole room indirect calorimeter built with walk-in cooler panels. This chamber and the reproducibility of the data generated have been previously described [15].

2.4. Metabolic chamber study day protocol

At approximately 0730 h subjects reported to the metabolic chamber following an overnight fast (no food or beverage from 2200 h). Subjects were instructed to avoid exercise the day before entering the chamber, and to not consume alcohol or caffeine during the two days prior to each study day. Before entering the chamber, subjects were weighed. At 0800 h, subjects were sealed in the chamber. Except for a 2-min interval each hour during which subjects were requested to stand and stretch, subjects were asked to remain seated or reclined, but awake throughout the study day. Subjects were asked to perform necessary daily activities such as using the restroom during these 2-min intervals where possible. Breakfast (at 0900 h), lunch (at 1330 h), and dinner (at 1700 h), were served through an air-lock passage. Subjects were instructed to finish each meal within 30 min. At 1400 h subjects were asked to place one of their arms through an iris port for blood sampling. Subjects were requested to prepare for bed at 2200 h, and at 2230 h, lights were turned off. Subjects were woken at 0630 h and at 0715 h, subjects exited the chamber and were weighed.

2.5. Irisin immunoprecipitations

Plasma samples (500 μg) were immunoprecipitated with one microgram anti-FNDC5 antibody (Acris Antibodies, San Diego CA) and 15 μL of a 50% slurry of protein A agarose CL-4B (Sigma, St. Louis MO) overnight at 4 °C. Agarose pellets were washed and irisin eluted with 25 μL Laemmli sample buffer. Irisin was resolved by SDS-PAGE (Bio-Rad, Hercules CA), transferred to nitrocellulose, blocked for 1 h and immunoblotted overnight at 4 °C with the same anti-FNDC5 antibody diluted 1:200 in Odyssey block buffer. The blot was washed and incubated for one hour at room temperature with goat anti-rabbit IgG IRDye 700DX antibody (Rockland Immunochemicals, Gilbertsville PA) diluted 1:10,000 in Odyssey block. After washing, bands were visualized on an Odyssey fluorescence imaging system (LI-COR, Lincoln NE). Irisin bands were quantitated using Odyssey software and integrated intensities were normalized to milli-litres plasma (II/ml). Linear regressions, goodness of fit (R^2) and p-values were determined with GraphPad Prism software. Plasma leptin levels were determined by a Luminex assay (EMD Millipore, Billerica, MA) and plasma adiponectin was assessed by an ELISA (Mercodia, Uppsala, Sweden).

3. Results

There was significant correlation between irisin levels and EE/kg FFM/h for subjects whose EE was greater than predicted by the FFM-based equation [418 + (20.3*FFM)] [13]. ($p = 0.0016$) (Fig. 1, open circles). In contrast, subjects whose EE was equal to or less than predicted by FFM exhibited no correlation with irisin levels (Fig. 1, closed circles). Furthermore, mean total 24 h measured EE was significantly higher (208 kcal) in the correlated (+C) group as compared with group for which there was no correlation (−C) (mean ± sd: 1808 ± 244 vs. 1600 ± 57; $p = 0.033$). There was no significant correlation between EE/kg/FFM and plasma levels of leptin (Fig. 2A) or adiponectin (Fig. 2B). No significant difference existed in age (60.9 ± 4.7 vs. 59.9 ± 5.9; $p = 0.68$) between the two sub-groups respectively. Additionally, groups were comparable for indices of body composition, with no significant differences observed in BMI (kg/m^2) (32.9 ± 5.9 vs.