EBioMedicine 2 (2015) 1725–1734

Contents lists available at ScienceDirect

EBioMedicine

journal homepage: www.ebiomedicine.com

Research Article

Risperidone-induced weight gain is mediated through shifts in the gut microbiome and suppression of energy expenditure

Sarah M. Bahr [a], Benjamin J. Weidemann [b], Ana N. Castro [a], John W. Walsh [a], Orlando deLeon [a], Colin M.L. Burnett [b], Nicole A. Pearson [b], Daryl J. Murry [c], Justin L. Grobe [b,e,f,g], John R. Kirby [a,d,e,*]

[a] Department of Microbiology, University of Iowa, Iowa City, IA, United States
[b] Department of Pharmacology, University of Iowa, Iowa City, IA, United States
[c] Department of Pharmaceutics and Translational Therapeutics, University of Iowa, Iowa City, IA, United States
[d] Department of Urology, University of Iowa, Iowa City, IA, United States
[e] Fraternal Order of Eagles' Diabetes Research Center, University of Iowa, Iowa City, IA, United States
[f] Obesity Research and Education Initiative, University of Iowa, Iowa City, IA, United States
[g] Center for Hypertension Research, University of Iowa, Iowa City, IA, United States

ARTICLE INFO

Article history:
Received 19 August 2015
Received in revised form 6 October 2015
Accepted 16 October 2015
Available online 20 October 2015

Keywords:
Risperidone induced weight gain, Gut microbiome, Suppression of energy expenditure
Suppression of energy expenditure
Phage transfer
Non-aerobic resting metabolic rate

ABSTRACT

Risperidone is a second-generation antipsychotic that causes weight gain. We hypothesized that risperidone-induced shifts in the gut microbiome are mechanistically involved in its metabolic consequences. Wild-type female C57BL/6J mice treated with risperidone (80 μg/day) exhibited significant excess weight gain, due to reduced energy expenditure, which correlated with an altered gut microbiome. Fecal transplant from risperidone-treated mice caused a 16% reduction in total resting metabolic rate in naïve recipients, attributable to suppression of non-aerobic metabolism. Risperidone inhibited growth of cultured fecal bacteria grown anaerobically more than those grown aerobically. Finally, transplant of the fecal phage fraction from risperidone-treated mice was sufficient to cause excess weight gain in naïve recipients, again through reduced energy expenditure. Collectively, these data highlight a major role for the gut microbiome in weight gain following chronic use of risperidone, and specifically implicates the modulation of non-aerobic resting metabolism in this mechanism.

1. Introduction

Recent studies associate the gut microbiome, the bacterial ecosystem that resides in the human gut, with the modulation of weight gain and metabolic diseases. It has been shown that the microbiome contributes to host metabolism and physiology by several mechanisms including increased energy harvest from the diet (Turnbaugh et al., 2006, 2008), modulation of lipid metabolism (Bäckhed et al., 2004; Velagapudi et al., 2010), altered endocrine function (Dumas et al., 2006; Swann et al., 2011; Wang et al., 2011), and inflammatory stability (Elinav et al., 2011; Hall et al., 2011; Henao-Mejia et al., 2012; Vandanmagsar et al., 2011). Thus the gut microbiota is influential in modulating obesity and other metabolic diseases.

Contributing to the obesity epidemic over the last two decades, the prescribing rate of second-generation antipsychotics for children has increased nearly eight-fold due to their efficacy (Findling et al., 2005). Second generation antipsychotics (SGAs) are used to treat a variety of psychiatric illnesses, including autism, bipolar disorder and schizophrenia. It has also been well established that the most commonly prescribed SGA, risperidone (a benzylamino-piperidine derivative), causes significant weight gain, insulin resistance, and metabolic syndrome (Möller et al., 2015; Calarge et al., 2012; Correll et al., 2009; De Hert et al., 2011). Numerous observational studies of risperidone-induced weight gain have led to significant advancements in treatment guidelines. However, the multifactorial nature of cardiometabolic effects is not completely understood, and patients taking risperidone still suffer from the side effects associated with weight gain. In humans, risperidone may promote weight gain through appetite stimulation, although many animal studies comparing SGAs challenge this hypothesis (Baptista et al., 2004; F. Li et al., 2013; Pouzet et al., 2003; Smith et al., 2012). Risperidone-induced weight gain is thought to be multifaceted, involving genetic, metabolic, and environmental contributors (Correll et al., 2011; Lett et al., 2011). Recent data implicates alterations in the gut microbiome as the mechanism by which SGAs impact metabolism and weight gain (Davey et al., 2013; Morgan et al., 2014). We have evidence that alterations in the gut microbiome of children chronically

* Corresponding author at: Department of Microbiology, 51 Newton Rd., 3-632 BSB, Iowa City, IA 52242, United States.
E-mail address: john-kirby@uiowa.edu (J.R. Kirby).

http://dx.doi.org/10.1016/j.ebiom.2015.10.018

treated with risperidone are associated with an increase in BMI compared to antipsychotic-naïve children (Bahr et al., 2015).

In this study, we examined the hypothesis that shifts in the gut microbiome are mechanistically linked to weight gain that occurs in response to risperidone treatment. We examined energy balance and weight gain in wild type mice in response to treatment with risperidone alone or in combination with various xenobiotics, transfer of risperidone-altered microbiota, or transfer of the phage associated with risperidone-treated microbiota. We found that risperidone alters the gut microbiota resulting in weight gain *via* suppressed energy expenditure. Furthermore, transfer of risperidone-treated fecal material, including the phage fraction alone, was sufficient to induce similar effects in naïve mice. Identification of the gut microbiome as a critical mediator of energy homeostasis may identify novel therapeutic targets and approaches for risperidone-induced weight gain and obesity.

2. Materials and methods

2.1. Animal husbandry

Six to seven-week old C57BL/6J female mice from the Jackson Laboratory were housed in a standard 12:12 dark–light cycle with *ad libitum* access to standard chow (Teklad 7013; 18% kcal from fat) and water (pH 3.7 with acetic acid to match the pH of oral risperidone) or water with risperidone (20 μg/ml, pH 3.7). Antibiotics were administered for ten days in drinking water at 62.5 mg/l and 142.5 mg/l of ciprofloxacin and ampicillin respectively. All procedures were approved by the University of Iowa Animal Care and Use Committee in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.2. Nuclear magnetic resonance

Body composition was assessed using nuclear magnetic resonance (NMR; Brucker LF90II). Mice were restrained (<1 min) for analysis and then returned to home cages.

2.3. Energy intake

Mice were individually housed in metabolic cages (Nalgene) to monitor fluid and food intake daily. Additionally, urine and fecal outputs were measured and collected. Fecal material was utilized to determine digestive efficiency by bomb calorimetry.

2.4. Bomb calorimetry

Caloric densities of desiccated food and fecal samples were determined using a 50 mg semi-micro bomb calorimeter (Parr). Energy absorption was calculated as:

$$[\text{Energy Absorbed}] = [\text{Energy Consumed}] - [\text{Fecal Energy}].$$

Energy Consumed is the product of the dry mass of food consumed and the caloric density of dry food, and Fecal Energy is the product of the dry mass of feces produced and the caloric density of the dried feces. Digestive efficiency was then calculated as:

$$[\text{Digestive Efficiency}] = \frac{[\text{Energy Absorbed}]}{[\text{Energy Consumed}]}.$$

Energy efficiency was calculated at various time points after the initiation of risperidone as:

$$[\text{Energy Efficiency}] = \frac{[\Delta \text{ Body Mass}]}{\left[\sum \text{Energy Absorbed}\right]}.$$

2.5. Physical activity & core temperature

Physical activity and core temperature were determined using radiotelemetric probes (DSI), as previously described (Burnett and Grobe, 2014; Grobe et al., 2010). Mice were anesthetized using isoflurane and probes were inserted inside the abdominal wall. Following 1–2 weeks of recovery, activity and core temperature were recorded for 30 s every 5 min throughout the light–dark cycle (Fig. S1).

2.6. Combined calorimetry

Resting metabolic rate (RMR) was measured simultaneously by direct calorimetry and respirometry as previously described (Burnett and Grobe, 2013, 2014). Briefly, rates of total heat dissipation, heat retention, and O_2 and CO_2 exchange of mice were assessed during sleep at thermoneutrality (30 °C) respectively, using a custom-built gradient-layer direct calorimeter, core temperature telemeters (DSI) and S-3A/II O_2 and CD-3A CO_2 analyzers (AEI). Mass flow of air was measured (Sensiron) and STP-corrected. Total RMR determined by direct calorimetry represents the sum of all heat dissipation (sensible and insensible) plus heat retained, which is determined by core temperature change and body composition as determined using NMR. Aerobic RMR determined by respirometry represents the estimated heat production using the formula derived from Lusk (1924):

$$[\text{Aerobic RMR}] = VO_2(1.232 \text{ RER} + 3.815).$$

VO_2 represents STP-corrected rate of oxygen consumption (in ml/min), and the respiratory exchange ratio, RER, represents the ratio of carbon dioxide production to oxygen consumption. Non-aerobic RMR represents the difference between measured total heat production (from direct calorimetry) and the estimated rate of aerobic RMR (from respirometry):

$$[\text{Total RMR}] = [\text{Aerobic RMR}] + [\text{Non}-\text{Aerobic RMR}]$$

It is important to note that throughout the current study, we report "RMR" data from mice, assessed while the animals are sleeping. The metabolic rate during sleep is more accurately referred to as "sleeping metabolic rate, SMR" but given the complexities of dissociating resting *vs.* sleeping metabolic rates in mice, few reports in the literature use this terminology in studies examining metabolic function in mice. In contrast, in humans SMR and RMR are clearly distinct. Thus although less technically accurate, we have chosen to report data herein as "RMR" instead of "SMR" as a reflection of the current vernacular of the field.

2.7. Bacterial DNA extraction and sequencing

16S rRNA gene sequencing methods were adapted from the methods developed for the NIH-Human Microbiome Project (Consortium, 2012). Briefly, bacterial genomic DNA was extracted using MO BIO PowerSoil DNA Isolation Kit (MO BIO Laboratories). The 16S rDNA V4 region was amplified by PCR and sequenced on the MiSeq platform (Illumina) using the 2 × 250 bp paired-end protocol yielding pair-end reads. The primers used for amplification contain adapters for MiSeq sequencing and dual-index barcodes so that the PCR products may be pooled and sequenced directly (Caporaso et al., 2012) targeting at least 6000 reads per sample.

2.8. Sequencing analysis

16S data was analyzed using QIIME v.1.9 software. Barcodes were matched to fastq files and then removed (Caporaso et al., 2010b). Similar sequences (cutoff 97%) were combined into operational taxonomic units (OTU) using sumaclust v1.1.00 and sortmerna 2.0.