Gastric Bypass Sensitizes Sympathetic and Thermogenic Activity of Brown Adipose Tissue to Cold Exposure

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Introduction

Multiple previous animal studies showed that Roux-en-Y gastric bypass (RYGB) induces an increase in total energy expenditure, specifically the resting metabolic rate [1–4]. It was recently demonstrated that this increase in resting metabolic rate is due to a sympathetically mediated increase in thermogenic activity of visceral white adipose tissue (vWAT) but not brown adipose tissue (BAT), at normal room temperature [5]. BAT activity has gained significant attention lately as a general marker of cardiometabolic health, and has been implicated in disorders such as hyperglycemia, hyperlipidemia, heart disease and even certain cancers [6]. Therefore, BAT activity might not be contributing to energy balance post-RYGB under normal daily conditions but might be engaged when temperature changes. To test this hypothesis, we examined the effect of RYGB on BAT sympathetic tone and thermogenic capacity upon cold exposure in diet-induced obese mice. To exclude weight as a confounding variable, we performed our studies in weight-matched sham-operated animals.

Method

RYGB and sham operations in mice were performed as previously described [1, 2].

In Vivo Recordings of Brown Adipose Tissue Sympathetic Nerve Activity (BAT SNA) to the Effects of Cooling in the RYGB- and Sham-Operated Mice

Mice anaesthetized with i.p. administration of ketamine (91 mg/kg body weight) and xylazine (9.1 mg/kg body weight) underwent intubation with PE-50 to provide an unimpeded airway for the mouse to spontaneously breathe O2-enriched room air. Next, a tapered micro-renathane tubing (MRE-40, Braintree Scientific) was inserted into the right jugular vein for infusion of the sustaining anesthetic agent, α-chloralose (initial dose: 12 mg/kg, then a sustaining dose of 6 mg/kg per h). Another tapered MRE-40 catheter was inserted into the left carotid artery for continuous measurement of arterial pressure and heart rate. Core body temperature was monitored with a rectal probe and continuously maintained at 37.5 °C with a temperature controller (Physitemp Model MCAT2). Each mouse was then equipped for direct multifiber SNA from the nerves serving the sub-scapular BAT. A bipolar platinum-iridium electrode (36-gauge, A-M Systems) was suspended under the nerve and secured with silicone gel (Kwik-Sil, WPI). The electrode was attached to a high-impedance probe (HIP-511, Grass Instruments), and the nerve...
were performed for this study at different temperature settings. Intraperitoneally with 8–μCi of [3H]-2-deoxyglucose in a 20% glucose solution, and tail blood was collected from all mice (time=0). All mice were then injected 30°C for 72 h. Following an overnight fast, tail blood was collected and homogenized in a lysis buffer (50 mM HEPES pH7.5, 150 mM NaCl, 1 mM MgCl2, 1 mM CaCl2, 10 mM NaF, 5 mM EDTA, 1% Triton X-100, 2 mM sodium orthovanadate, and Roche cocktail protease inhibitor tablet). Protein concentrations were measured, and equal amounts were loaded to a 8% SDS PAGE gel for electrophoresis. Proteins on the gel were electro-transferred to a PVDF membrane (Bio-Rad). The blots were then blocked with 5% (w/v) non-fat milk in TBST (0.1% Tween 20) and were probed with anti-TH (2025-THRAB, 1:600, Santa Cruz) overnight at 4°C. After washing with TBST, the blots were incubated with a secondary anti-rabbit or anti-mouse IgG antibody (1:10,000) for 2 h. After washing with TBST, protein of interest was imaged with an enhanced chemiluminescence (ECL) detection kit (GE Healthcare, Little Chalfont, UK) on an imager.

Statistical Analysis

All reported data are presented as mean ± standard error of means (SEM) with statistical analysis by Student’s t test or one-way ANOVA that was followed by the Tukey-Kramer correction analysis if necessary. Figures were generated using GraphPad Prism 9.0 (GraphPad Software), and a p value of < 0.05 was considered statistically significant.

Results

We report that cooling results in a significant increase of BAT sympathetic nerve activity (SNA) in RYGB-operated mice at peak-cold temperature. There was a 40.5% increase in SNA intensity (measured by RVI; V*s/min) and 104% increase in SNA velocity (measured by frequency spikes/s) (p=0.0011). On the other hand, re-heating to a normal core body temperature of 37 °C reverses this RYGB-mediated augmentation in SNA (Fig. 1a, b). It is also important to note that this bypass-induced plasticity of sympathetic nerve fibers is independent of surgical-induced weight loss as animals were tested 1-week post-surgery where sham- and RYGB-operated mice were weight-matched (Fig. 1c). Additionally, the cooling did not cause any significant changes in mean arterial blood pressure or heart rate between the two groups (Fig. 1d). This further suggests that this SNA modulation is not due to systemic non-specific activation of the whole autonomic/sympathetic axis.

Moreover, and consistent with the above SNA experiment, cold exposure to a core temperature of 27–28 °C induces thermogenic surge in BAT expression of TH and UCP1 post-RYGB furthermore reinforcing our sympathetic neurogram data above (Fig. 2a). Likewise, 2-deoxyglucose uptake was increased by BAT in RYGB-operated mice compared to their sham counterparts (p=0.038) when experiment was performed under cold but not warm temperature conditions (Fig. 2b).
Fig. 1 Increased sensitivity of brown adipose tissue (BAT) sympathetic nerve activity (SNA) to cooling after Roux-en-Y gastric bypass. a Percent change in SNA of BAT (expressed in RVI, \(^{\circ}\)C/min (top panel) and in spikes/s (lower panel)) to cooling and heating effect between sham- and RYGB-operated mice, 1 week after surgery. b Representative neurogram of BAT sympathetic nerve activity in sham- and RYGB-operated mice at peak hot and peak cool temperature. c Body weight of sham- and RYGB-operated mice at week 1 after surgery. d Change in mean arterial pressure (MAP) and heart rate (HR) in sham- and RYGB-operated mice during the cooling/heating experiment. One-way ANOVA was used to detect difference between the two groups in a. *, \(p < 0.05\). Sham, n=8; RYGB n=6.

Fig. 2 Increase in thermogenic activity of BAT after Roux-en-Y gastric bypass. a BAT protein expression of TH and UCP1 by western blot in sham- and RYGB-operated mice sacrificed at core temperature of 37–38 °C (warm exposure) or at 27–28 °C (cold exposure). b Radiolabeled \([\text{H}]\)-2-deoxyglucose uptake in nmol/g/min by BAT in sham- and RYGB-operated mice 1 week after surgery at 30 °C temperature (warm exposure) and at 20 °C temperature (cold exposure). Student t-test was used to detect difference between the two groups in b. *, \(p < 0.05\). Sham, n=6-8; RYGB n=8.
Discussion

Here we report that RYGB, one of the most effective and commonly performed bariatric procedures, causes selective activation of autonomic sympathetic activation of brown adipose tissue to induce thermogenesis under cold conditions in a weight-independent manner. We previously showed that browning of white adipose is responsible for baseline increase in thermogenic activity and increased energy expenditure post-RYGB under room temperature conditions [5]. Our recent findings suggest that BAT could also contribute to a further augmentation of energy expenditure induced by RYGB, resulting in its long-term efficacy as a weight loss procedure. Increased activity of BAT has also been tightly associated with improvement of several cardiometabolic outcomes in animals and humans [6, 9]. Enhancing the sensitivity of BAT activity to cold exposure may also contribute to the other cardiometabolic benefits of RYGB.

Being able to understand the underlying regulators of BAT activity in normal physiology (as well as in the setting of bariatric surgery) could help with future development of BAT-targeted therapy that can further improve our current management of obesity and its associated metabolic disorders. In conclusion, our data show that BAT activity is plastic post-RYGB and is independent of surgical-induced weight loss. This bypass-mediated augmentation in BAT activity upon cold exposure might be accountable for the day-to-day variations in thermogenic activity as well as the other metabolic benefits of bariatric surgery.

Declarations

Conflict of Interest The authors declare no competing interests.

Statement of Ethical Declaration All applicable institutional and/or national guidelines for the care and use of animals were followed.

Informed Consent Statement Informed consent does not apply.

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