Brief communication

MOLECULAR METABOLISM

Activation of natriuretic peptides and the sympathetic nervous system following Roux-en-Y gastric bypass is associated with gonadal adipose tissues browning

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ABSTRACT

Objective: Roux-en-Y gastric bypass (RYGB) is an effective method of weight loss and remediation of type-2 diabetes; however, the mechanisms leading to these improvements are unclear. Additionally, adipocytes within white adipose tissue (WAT) depots can manifest characteristics of brown adipocytes. These 'BRITE/beige' adipocytes express uncoupling protein 1 (UCP1) and are associated with improvements in glucose homeostasis and protection from obesity. Interestingly, atrial and B-type natriuretic peptides (NPs) promote BRITE/beige adipocyte enrichment of WAT depots, an effect known as "browning." Here, we investigate the effect of RYGB surgery on NP, NP receptors, and browning in the gonadal adipose tissues of female mice. We propose that such changes may lead to improvements in metabolic homeostasis commonly observed following RYGB.

Methods: Wild type, female, C57/Bl6 mice were fed a 60% fat diet *ad libitum* for six months. Mice were divided into three groups: Sham operated (SO), Roux-en-Y gastric bypass (RYGB), and Weight matched, sham operated (WM-SO). Mice were sacrificed six weeks following surgery and evaluated for differences in body weight, glucose homeostasis, adipocyte morphology, and adipose tissue gene expression.

Results: RYGB and calorie restriction induced similar weight loss and improved glucose metabolism without decreasing food intake. β3-adrenergic receptor expression increased in gonadal adipose tissue, in addition to *Nppb* (BNP), and NP receptors, *Npr1*, and *Npr2*. The ratio of *Npr1*:*Npr3* and *Npr2*:*Npr3* increased in RYGB, but not WM-SO groups. *Ucp1* protein and mRNA, as well as additional markers of BRITE/beige adipose tissue and lipolytic genes increased in RYGB mice to a greater extent than calorie-restricted mice.

Conclusions: Upregulation of *Nppb*, *Npr1*, *Npr2*, *and β3-adrenergic receptors* in gonadal adipose tissue following RYGB was associated with increased markers of browning. This browning of gonadal adipose tissue may underpin the positive effect of RYGB on metabolic parameters and may in part be mediated through upregulation of natriuretic peptides.

Keywords High fat diet (HFD); Roux-en-Y gastric bypass (RYGB); Natriuretic peptide receptor; Atrial natriuretic peptide (ANP); B-type natriuretic peptide (BNP); Browning

1. INTRODUCTION

Obesity and its associated co-morbidities are international public health concerns. As the prevalence of obesity has increased over the past decade, it has become clear that bariatric surgery can be more effective than diet and lifestyle interventions for weight loss and remediation of obesity-related morbidities [1–4]. Roux-en-Y gastric bypass (RYGB) is the most commonly performed bariatric surgery worldwide and involves physical reconstruction of the gastrointestinal tract [5]. In RYGB, a small stomach pouch is formed by ligation of the proximal from the distal stomach. The jejunum is then separated from the distal duodenum and attached to the newly created stomach pouch. Ingested food moves quickly from the stomach pouch into the new jejunal limb; thus, food bypasses a significant portion of proximal small intestine [5]. RYGB produces rapid initial weight loss, which, unlike conventional diet-induced weight loss, can be maintained for years after surgery, as well as improvements in diabetes and cardiovascular health [6,7]. Interestingly, reductions in caloric intake following RYGB do not fully account for the magnitude of weight loss commonly seen after the surgery

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[6,8]. This suggests that molecular mechanisms affecting metabolism and nutrient utilization underlie the effect of RYGB. Understanding the nature of these mechanisms is essential to developing novel, non-surgical obesity interventions.

Human and rodent adipose tissues comprise several distinct adipocyte sub-types [9–11]. White adipocytes are the prototypical fat cell, capable of storing a large amount of lipid and secreting a wide variety of cytokines. Brown adipocytes are found in depots discrete from white adipose tissue (the interscapular depot in mice and supraclavicular and paraspinal depots in humans [10]), have more mitochondria, and constitutively express uncoupling protein 1 (UCP1) [12,13]. Expression of *Ucp1* conveys a thermogenic function to brown adipocytes; rather than oxidizing substrates to produce ATP, cellular respiration in brown adipocytes results in heat production [14]. A third sub-type, Brown-in-white (BRITE)/beige adipocytes, arises within white adipose tissue depots from the same progenitor population as white adipocytes but functionally resembles brown adipocytes [12]. Both brown and beige adipocytes express *Ucp1* [15,16]; in both cell types thermogenic activity can be induced by exposure to cold temperatures, sympathetic nervous system (SNS) signaling (such as β-adrenergic agonists), and the cardiac natriuretic peptides, namely, atrial natriuretic peptide (ANP) and B-type natriuretic peptide (BNP) [17]. Interestingly, it has been shown that RYGB surgery elevates cardiac natriuretic peptide expression in humans [18].

Brown and beige adipocytes provide beneficial whole-body and tissue-specific metabolic effects beyond thermal insulation. For example, beige adipocyte enrichment following treatment with β3-adrenergic agonists improves glucose uptake in insulin resistant subcutaneous and visceral white adipose depots in mice [19], and the amount of brown adipose tissue correlates inversely with body mass index (BMI) and fasting glucose in humans [11]. Since known inducers of beige adipocyte activity are elevated following RYGB surgery, it makes sense to investigate whether beige-enrichment of WAT depots follows RYGB and whether these inducible beige enrichments are associated with improved metabolic health in a weight-loss independent manner. The purpose of the present study was to determine whether changes in energy metabolism and glucose homeostasis after RYGB surgery result, in part, from beige enrichment due to upregulation of NPs and altered SNS signaling. Importantly, approximately 80% of all bariatric surgeries in the United States are performed on women [20]. As such, we chose to examine the effects of RYGB on beige enrichment in female murine adipose tissues.

Here, we report for the first time, increased levels of natriuretic peptide receptors 1 and 2 (*Npr1*, *Npr2*) and B-type natriuretic peptide (*Nppb*, *aka BNP*) mRNA, as well as increased levels of β3-adrenergic receptors and lipolytic genes in the gonadal adipose tissue of RYGB mice. Furthermore, we recognize a general pattern of gene expression and adipocyte morphology in gonadal adipose tissue that strongly suggests beige enrichment. Our data suggest the positive effect of RYGB on metabolic parameters may in part be mediated through upregulation of natriuretic peptides but do not exclude the possibility that other mechanisms may be involved. This evidence supports the hypothesis that improvements in metabolism seen following RYGB surgery are driven by mechanisms beyond weight loss per se.

2. MATERIAL AND METHODS

2.1. Animal care

Studies were conducted in accordance with University of Texas Southwestern Medical Center (UTSW) Institutional Animal Care and Use Committee and the Association of Assessment and Accreditation of Laboratory Animal Care policies. Mice were individually housed in a temperature-controlled environment at 22°C–24 °C using 12-h light/12-h dark cycles (Light cycle: 0600–1800).

2.2. Study design

Female C57BL/6 mice were placed on high fat diet (HFD) (D12492, Research Diets) from 6 weeks of age. Upon reaching approximately 40–45 g (12–14 weeks on HFD), mice were randomized to receive Roux-en-Y gastric bypass surgery (RYGB) or sham operations (SO). To control for the effects of weight loss per se an additional subset of female sham-operated diet-induced obese mice were weight-matched to the RYGB group by calorie restriction (WM-SO). After recovery, SO and RYGB mice were provided HFD *ad libitum*. Body weight was monitored daily and body composition evaluated using a Minispec mq10 NMR (Bruker Optics) prior to sacrifice. Food intake was measured over four consecutive days during post-operative week four. Oral glucose tolerance test and insulin tolerance tests were conducted during post-operative week six. Animals were sacrificed at post-operative week six.

2.3. Surgical intervention

RYGB surgery was performed as described [21]. Briefly, RYGB involved gastrointestinal reconstruction such that ingested nutrients pass from a proximal gastric pouch into a jejunal afferent limb. Distal stomach and proximal intestine were excluded from alimentary flow using a vascular clip (Ethicon) placed just distal to the gastro-jejunostomy. The sham procedure involved gastrotomy, enterotomy, and repair. Mice were anesthetized using a scavenged circuit of isoflurane; anesthesia time was standardized between groups. Mice were maintained on a post-operative feeding protocol during which liquid diet was provided from post-operative days two through seven. On post-operative day six, 0.25 g of HFD was provided on a daily basis until consumed in its entirety. Subsequently, solid diet was re-introduced *ad libitum*. WM-SO mice were provided food once a day at the onset of the dark phase. The amount of food given to the WM-SO group was adjusted in order to induce weight loss equal to that of the RYGB group.

2.4. Oral glucose tolerance test (OGTT)

SO and RYGB mice were fasted for three hours (beginning two hours into light cycle) prior to administration of glucose (1 g/kg body weight; Sigma–Aldrich) by gavage. Importantly, WM-SO mice were fed at the onset of the dark phase and regularly consumed all food within one hour; therefore, at the time of OGTT this group had been without food for a longer duration than the other groups. Mice did not have access to food throughout the experiment. At the indicated time points, blood samples were collected in heparin-coated capillary tubes from the tail vein and assayed as described in the Hormone and metabolite measurements section.

2.5. Insulin tolerance test (ITT)

Mice were fasted for three hours (beginning two hours into light cycle) prior to administration of insulin (0.75 U/kg body weight; Eli Lilly) via intraperitoneal injection. Importantly, WM-SO mice were fed at the onset of the dark phase and regularly consumed all food within one hour; therefore, at the time of OGTT this group had been without food for a longer duration than the other groups. Mice did not have access to food throughout the experiment. At the indicated time points, blood samples were collected in heparin-coated capillary tubes from the tail vein and assayed as described in the Hormone and metabolite measurements section.

