Original Article

MOLECULAR METABOLISM

Therapeutic reduction of lysophospholipids in the digestive tract recapitulates the metabolic benefits of bariatric surgery and promotes diabetes remission

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ABSTRACT

Objective: Obesity and obesity-related metabolic disorders are major health problems worldwide. The most effective obesity intervention is bariatric surgery. This study tested the hypothesis that bariatric surgery alters phospholipid metabolism in the gastrointestinal tract to favor a metabolically healthy gut microbiota profile and therapeutic intervention of phospholipid metabolism in the gastrointestinal may have similar metabolic benefits.

Methods: The first study compared plasma levels of the bioactive lipid metabolites lysophospholipid and trimethylamine N-oxide (TMAO) as well as gut microbiota profile in high fat/carbohydrate (HFHC) diet-fed C57BL/6 mice with or without vertical sleeve gastrectomy (VSG) and in *Pla2g1b*$^{-/-}$ mice with group 1B phospholipase A_2 gene inactivation. The second study examined the effectiveness of the non-absorbable secretory phospholipase A_2 inhibitor methyl indoxam to reverse hyperglycemia and hyperlipidemia in HFHC diet-fed C57BL/6 mice after diabetes onset.

Results: Both bariatric surgery and PLA2G1B inactivation were shown to reduce lysophospholipid content in the gastrointestinal tract, resulting in resistance to HFHC diet-induced alterations of the gut microbiota, reduction of the cardiovascular risk factors hyperlipidemia and TMAO, decreased adiposity, and prevention of HFHC diet-induced diabetes. Importantly, treatment of wild type mice with methyl indoxam after HFHC diet-induced onset of hyperlipidemia and hyperglycemia effectively restored normal plasma lipid and glucose levels and replicated the metabolic benefits of VSG surgery with diabetes remission and TMAO reduction.

Conclusion: These results provided pre-clinical evidence that PLA2G1B inhibition in the digestive tract may be a viable alternative option to bariatric surgery for obesity and obesity-related cardiometabolic disorder intervention.

Keywords Phospholipase A_2; Gut microbiota; Cardiometabolic disease; Bariatric surgery; Lysophospholipid

1. INTRODUCTION

The increasing prevalence of obesity and its associated cardiometabolic diseases has limited successful treatment options, thereby demanding the discovery of novel intervention strategies to combat these debilitating disorders and reduce the burden of health cost. The current strategy for obesity management includes lifestyle modifications, pharmacologic therapy, and bariatric surgery [1]. Lifestyle modification is usually the first line of intervention but is generally ineffective due to high relapse rates. Pharmacotherapy for obesity has been disappointing so far, and some of the obesity drugs have been associated with serious side effects [2]. The most effective obesity intervention currently is bariatric surgery [2]. Because of potential risks associated with surgical procedures, the development of novel therapeutics that can mimic the metabolic benefits of bariatric surgery is highly desirable [3,4]. A critical barrier to achieving this goal is the insufficient knowledge of the mechanisms underlying the metabolic benefits of bariatric surgery.

The most commonly practiced bariatric procedures are the Roux-en Y gastric bypass and vertical sleeve gastrectomy (VSG). The metabolic benefits of both procedures have been attributed to a combination of nutrient transport bypassing the proximal intestine with rapid delivery to the distal intestine, and altering enterohepatic circulation to increase circulating bile acids that improve insulin sensitivity [5–9]. Another

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Abbreviations: HFHC, High fat high carbohydrate; LPC, Lysophosphatidylcholine; LPCAT3, Lysophosphatidylcholine acyltransferase-3; PLA2G1B, Group 1B phospholipase A_2; TMA, Trimethylamine; TMAO, Trimethylamine N-oxide; TMSP, 3-Trimethylsilyl 2,2,3,3-d_4 propionate; VSG, Vertical sleeve gastrectomy

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mechanism underlying the metabolic benefits of bariatric surgery is changes in the gut microbiota, which can alter energy yield from food and affect several nutrient metabolism pathways in the distal small intestine and colon [10]. The relationship between bariatric surgery and gut microbiota changes leads to the hypothesis that manipulating the gut microbiota may be a viable strategy to mimic the metabolic benefits of bariatric surgery [11,12].

Current approaches aimed at gut microbiota manipulation include dietary modification, prebiotic/probiotic/symbiotic supplementation, and fecal microbiota transplant [12]. Unfortunately, dietary modification has high relapse rate while prebiotic and probiotic supplementation may cause unexpected weight gain [13]. Although fecal microbiota transplant shows promising results, the gut microbiota can adapt to environment dictated by the diet that is characteristic of metabolic dysfunction phenotype within hours and days after high fat feeding [14–16]. Thus, repeated fecal microbiota transplant is necessary to achieve sustained metabolic benefits, which adds to social and ethical concerns that limit its long term use for chronic metabolic disease management [17]. Accordingly, the development of pharmacotherapy that can mimic the effect of bariatric surgery on gut microbiota is critical for effective management of the global public health crisis associated with obesity and obesity-related metabolic diseases [18].

A recent survey of metabolomic and lipidomic changes associated with bariatric surgery in humans revealed that successful diabetes remission is associated with reduced sphingomyelin and lysophospholipid levels [19]. In view of previous studies showing that reducing intestinal phosphatidylcholine hydrolysis to lysophosphatidylcholine (LPC) via group 1B phospholipase A_2 (PLA2G1B) inhibition suppresses the onset of diet-induced obesity and hyperglycemia in mice [20,21], the goals of this study is to determine whether PLA2G1B inactivation also alters gut microbiota, and more importantly whether PLA2G1B inhibitors may be a viable therapeutic option instead of bariatric surgery for diabetes intervention to reverse hyperglycemia, hyperinsulinemia, and hyperlipidemia after their onset.

2. METHODS

2.1. Animals

Wild type C57BL/6J mice purchased from Jackson Laboratories (Bar Harbor, ME) were used for all bariatric surgery experiments. For experiments with *Pla2g1b*$^{-/-}$ mice, the *Pla2g1b*$^{-/-}$ mice were backcrossed with C57BL/6J mice originally obtained from Jackson Laboratories for >10 generations to generate *Pla2g1b*$^{-/-}$ mice in C57BL/6J background [22]. Breeding colonies of *Pla2g1b*$^{+/+}$ and *Pla2g1b*$^{-/-}$ mice were maintained in our institutional animal facility and littermates were used for all experiments. All animals were maintained in a temperature- and humidity-controlled room with a 12-hr light/dark cycle. The animals were maintained on rodent chow (LM485; Harlan-Teklad, Madison, WI) with free access to water. Male mice at the age of 12–14 weeks were used for all experiments except for the cohousing studies when 2 female mice placed in the same cage were studied due to aggressive nature of male mice. The animals were fed either the rodent chow or a high fat-high carbohydrate (HFHC) diet (D12331; Research Diets, New Brunswick, NJ). In selected experiments, the mice were administered drinking water containing the antibiotics vancomycin (0.5 g/L) and bacitracin (1 g/L) during the 8-week HFHC dietary feeding period as described [23]. All experimental animal protocols were approved by the institutional animal care and use committee at the University of Cincinnati in accordance with the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health.

2.2. PLA2G1B inhibition studies

The pan-secretory phospholipase A_2 inhibitor methyl indoxam, which is cell impermeable and cannot be absorbed through the intestinal mucosa [21], was used to test the effect of PLA2G1B inhibition on metabolic diseases. Methyl indoxam was synthesized by Intogen Biosciences (Telangana, India) according to the procedure described [24]. After gas chromatography confirmation of >95% purity and in vitro inhibition of PLA2G1B activity, the methyl indoxam was added to the HFHC diet at a concentration to yield a dosage of 90 mg kg^{-1}. Body weights were recorded at the beginning as well as throughout the experimental feeding period. Body fat mass was measured in conscious mice using proton magnetic resonance spectroscopy (EchoMRI-100; Echo Medical Systems).

2.3. Vertical sleeve gastrectomy surgery

VSG surgery was performed in C57BL/6J mice after feeding the HFHC diet to induce obesity and glucose intolerance as described [25]. Briefly, the mice were anesthetized with isofluorane inhalation. The lateral 80% of the stomach was excised leaving a tubular gastric remnant in continuity with the esophagus superiorly and the pylorus and duodenum inferiorly. A sham procedure without stomach excision was performed similarly with manual pressure along a vertical line between esophageal sphincter and the pylorus. The mice were fed Osmolite One Cal liquid diet one day before surgery for acclimation and then three days after the operation prior to re-introduction of the HFHC diet. Food consumption was assessed daily for 4 consecutive days 3 weeks after the recovery period and was repeated again 3 weeks later as described [26]. Intestinal expression of lysophosphatidylcholine acyltransferase-3 (LPCAT3) was assessed by isolating total RNA from the ileum for RT-PCR quantification of its mRNA. The primers used for amplification of LPCAT3 mRNA are: 5′-CCAGGGAAGATGCCAAACAG-3′ and 5′-GTGTAGCCCACCAGGTAGACAAG-3'. Cyclophilin mRNA amplification with primers: 5′-TCATGTGCCAGGGTGGTGAC-3′ and 5′-AACTTCAGTCTTGGCAGTGC-3′ was used as the normalization control.

2.4. Postprandial glucose tolerance test

Mice were fasted overnight and then fed 4 ml-kg^{-1} body weight of a bolus HFHC meal containing 2.6 mM egg phosphatidylcholine, 13.33 mM triolein, and 2.6 mM cholesterol in a saline solution containing 50% w/v glucose. Blood was obtained before and at different times after administration of the test meal. Blood glucose levels were measured with an Accu-Chek glucometer (Roche Applied Science, Indianapolis, IN).

2.5. Plasma chemistry

Animals were fasted overnight prior to blood draw for plasma chemistry measurements. Plasma cholesterol and triglyceride levels were measured using the Infinity Triglyceride and Cholesterol Assay Kits from Thermo Fisher Scientific (Middletown, NJ). Glucose levels were measured with an Accu-Chek glucometer, and insulin levels were determined by the UltraSensitive Rat Insulin ELISA kit (Crystal Chem, Chicago, IL). Homeostatic model assessment of insulin resistance was calculated from fasting glucose and insulin levels using the formula [glucose (mg/dL) x insulin (ng/ml)]/22.5.

2.6. Lysophosphatidylcholine assays

Plasma LPC concentrations were determined by enzymatic assay as described [22]. To measure LPC content in the digestive tract, the entire small intestine was collected and flushed with phosphate-buffered saline and then flash frozen at −80 °C. The intestine was thawed in buffer containing 50 mM Tris-Cl, pH 7.4, 150 mM NaCl, and 10 mM EDTA.

