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Basic nutritional investigation

Sodium butyrate modulates adipocyte expansion, adipogenesis, and insulin receptor signaling by upregulation of PPAR-γ in obese Apo E knockout mice

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ABSTRACT

Objectives: Studies suggest that sodium butyrate reduces obesity-associated inflammation and insulin resistance in in vitro and in vivo models. Apo $E^{-/-}$ mice have high basal oxidative stress and naturally develop dyslipidemia and atherosclerosis. Because these disorders are present in obesity, the aim of this study was to determine whether Apo $E^{-/-}$ mice could be a more realistic model for studying obesity and insulin resistance.
Methods: We evaluated the action of orally administered sodium butyrate on adipose tissue expansion and insulin resistance using diet-induced obese Apo $E^{-/-}$ mice.
Results: Findings from the present study demonstrated that obese mice fed a sodium butyrate–supplemented diet presented a modest reduction of weight gain associated with reduction of adipocyte expansion, induction of adipogenesis and angiogenesis, and adiponectin production. Sodium butyrate also improved insulin sensitivity, by increasing insulin receptor expression associated with activation of Akt signaling pathway. These results were associated with increased peroxisome proliferator-activated receptor-γ expression and nuclear factor-κB downregulation.
Conclusion: These results suggested that oral supplementation of butyrate could be useful as an adjuvant in the treatment of obesity, metabolic syndrome, and insulin resistance.

Introduction

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder that is growing at an alarming rate as global obesity rates increase. In addition to lifestyle, metabolic factors are involved in the pathogenesis of T2DM. In a simplistic way, the linking between obesity and insulin resistance (IR) starts with the hypertrophy of adipocytes and adipose expansion. This induces adipocyte stress, macrophage infiltration, and inflammation by a mechanism involving the nuclear factor (NF)-κB pathway. Activation of macrophages and adipocytes triggers dysregulated adipokine release (like adiponectin downregulation and leptin upregulation), which impairs insulin signaling and glucose transporter type 4 (GLUT4) translocation, leading to IR in adipose tissue and other peripheral organs [1,2].

Butyrate is a 4-carbon short-chain fatty acid that reduces inflammation through several mechanisms, including reduction of immune cell infiltration, secretion of proinflammatory cytokines, and production of antioxidant agents [3–5]. These activities are primarily associated with the ability of butyrate to inhibit the activity of histone deacetylase (HDAC), which removes acetyl moieties from chromatin structures [6]. Acetyl

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moieties relax the chromatin and facilitate access of transcription factors to target gene promoters. A positive association between HDAC activity and IR has been previously described [7,8] and is related to the reduction of GLUT4-mediated glucose uptake in the skeletal muscle and liver [8]. Thus, HDAC inhibitors such as butyrate could improve the control of IR.

Previous studies suggest that butyrate can reduce weight gain, energy expenditure, obesity-associated inflammation, and IR in both in vitro and in vivo models [7,9]. Each one of these studies analyzed different mechanisms in different organs of different animal models. Apolipoprotein (Apo)E knockout (KO) mice have a high basal level of oxidative stress and naturally develop dyslipidemia and atherosclerosis [10–13]. As dyslipidemia, atherosclerosis, and oxidative stress usually are present in patients with obesity, we believe that ApoE KO mice are a more realistic model than wild-type C57 BL/6 mice for studying IR related to diet-induced obesity. Therefore, we evaluated the action of orally administered sodium butyrate (NaB) on adipose tissue expansion and glucose homeostasis in the adipose tissue of ApoE KO mice that were fed a high-fat diet.

Methods

Animals and diets

Six-wk-old male ApoE KO mice were group-housed in an environment with controlled light and temperature. The animals were divided into four groups that were fed different experimental diets for 10 wk: Control (CT) and control + butyrate (CT+BUT) groups received a standard diet (66% carbohydrate, 8% fat, and 20% protein, v/v); obese (OB) and obese + butyrate (OB+BUT) groups received a high-fat diet (25% carbohydrate, 60% fat, and 15% protein, v/v) [12]. In the BUT groups, diets were supplemented with 1% NaB (10 mL/kg diet) using 10 mL of butyric acid (Sigma-Aldrich Co., St. Louis, MO, USA) adjusted with 4 N NaOH to pH 7.2 [4]. All mice had free access to food and water.

Body weight and food intake were measured individually once a week. Weight gain was calculated by the difference between the final and initial weight of the animals. At the end of the experiment, the animals were fasted overnight and sacrificed under anesthesia. Blood and epididymal fat were collected for subsequent analyses.

Adiposity and histologic analyses

Epididymal adipose tissue was dried and weighed to calculate its absolute and relative weight (expressed as a percentage of body weight). For the analysis of adipocyte size, tissue was cut into 7-μm sections, and slides containing the samples were stained with hematoxylin and eosin (HE). After image capture (100× magnification), 100 adipocytes were measured for each animal using Image Pro-Plus software (Bethesda, MD, USA). The number of adipocytes in each image (three per animal) was counted to determine the number of adipocytes per field.

Glucose homeostasis

Blood glucose was determined using commercial kits (Labtest, Lagoa Santa, Brazil). Oral glucose tolerance and insulin sensitivity tests were performed as previously described [14]. Serum adiponectin was measured according to the manufacturer's instructions (BD Bioscience Pharmigen, San Diego, CA, USA).

Determination of matrix metalloproteinases 2 and 9 activities

The gelatinolytic activity of matrix metalloproteinases (MMP)2 and MMP9 was identified by electrophoresis in the presence of sodium dodecyl sulfate (SDS) in polyacrylamide gels (PAGE) containing 1 mg/mL gelatin [15]. Briefly, adipose tissues were lysed in homogenization buffer (Tris-base 50 mM, triton X-100 0.5%; pH 7.5) and loaded on gels (60 μg of protein) after centrifugation (5000g/10 min, 4°C). After electrophoresis (2 h), proteins were renatured by exchanging SDS with 2.5% Triton X-100 (30-min incubation repeated twice). The gels were then incubated for 18 h at 37°C in 50 mM Tris-HCl, pH 7.5, 5 mM $CaCl_2$, and 0.02% Brij35, followed by staining with Coomassie blue. The gelatinolytic

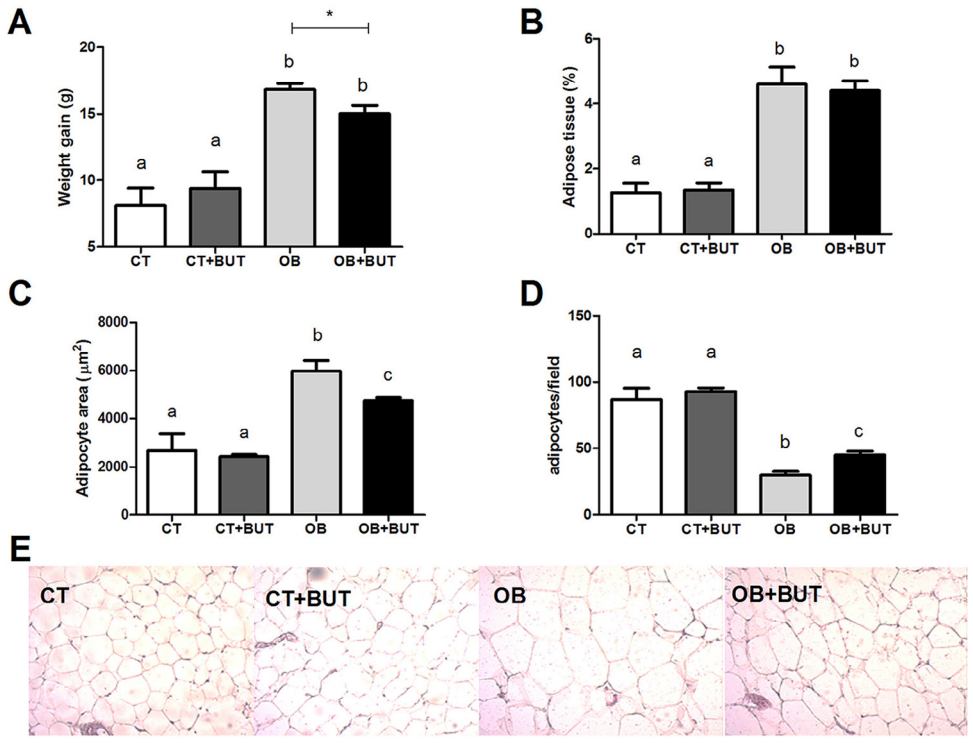

Fig. 1. Sodium butyrate reduces weight gain and adipocyte expansion in obese mice. (**A**) Body weight gain. (**B**) Relative epididymal adipose tissue (as percent of weight body). **C,** Adipocyte area. (**D**) Number of adipocytes per field of mice fed control (CT) or high-fat (OB) diet with or without butyrate supplementation (BUT) for 10 wk. (**E**) Adipose tissue histology (hematoxylin and eosin). Control (CT), control + butyrate (CT+BUT), obese (OB), obese + butyrate (OB+BUT). Mean values not sharing the same letters are significantly different, $P < 0.05$. n = 9 to 11 mice/group.