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Effects of sodium benzoate, a widely used food preservative, on glucose homeostasis and metabolic profiles in humans

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ABSTRACT

Sodium benzoate is a widely used preservative found in many foods and soft drinks. It is metabolized within mitochondria to produce hippurate, which is then cleared by the kidneys. We previously reported that ingestion of sodium benzoate at the generally regarded as safe (GRAS) dose leads to a robust excursion in the plasma hippurate level [1]. Since previous reports demonstrated adverse effects of benzoate and hippurate on glucose homeostasis in cells and in animal models, we hypothesized that benzoate might represent a widespread and underappreciated diabetogenic dietary exposure in humans. Here, we evaluated whether acute exposure to GRAS levels of sodium benzoate alters insulin and glucose homeostasis through a randomized, controlled, cross-over study of 14 overweight subjects. Serial blood samples were collected following an oral glucose challenge, in the presence or absence of sodium benzoate. Outcome measurements included glucose, insulin, glucagon, as well as temporal mass spectrometry-based metabolic profiles. We did not find a statistically significant effect of an acute oral exposure to sodium benzoate on glucose homeostasis. Of the 146 metabolites targeted, four changed significantly in response to benzoate, including the expected rise in benzoate and hippurate. In addition, anthranilic acid, a tryptophan metabolite, exhibited a robust rise, while acetylglycine dropped. Although our study shows that GRAS doses of benzoate do not have an acute, adverse effect on glucose homeostasis, future studies will be necessary to explore the metabolic impact of chronic benzoate exposure.

1. Introduction

Sodium benzoate and potassium benzoate are commonly used food preservatives that are listed among the "generally regarded as safe" (GRAS) compounds by the United States Food and Drug Administration, and can be present in foods at a concentration up to 0.1%. Though present in a variety of foods, population studies indicate that soft drinks are the major dietary source of benzoate [2]. Dietary benzoate is conjugated to glycine to form hippurate in the liver and kidneys by a two-step reaction occurring in the mitochondrial matrix (Fig. 1) [3], so ingestion of this preservative causes a rise in both serum benzoate and hippurate levels [4]. Benzoate metabolism within mitochondria results in the consumption of ATP and glycine, as well as transient sequestration of coenzyme A (CoA). This compound can therefore have significant metabolic effects, as was demonstrated by early studies showing a drop in serum glycine with a high oral dose of sodium benzoate [5,6]. In fact, indirect consumption of waste nitrogen through disposal of glycine is the rationale behind using intravenous sodium benzoate to treat hyperammonemia in patients with urea cycle disorders [7], and to reduce glycine levels in patients with non-ketotic hyperglycinemia. The clinically used dose of sodium benzoate can also be associated with other metabolic disturbances, such as a carnitine deficiency due in part to increased excretion of benzoylcarnitine [8,9], and even higher doses have been associated with hepatic ATP depletion in animal studies [10]. Of course, the intravenous dose of sodium benzoate used in these conditions is 250 mg/kg/day, a much higher exposure than the 6.3 mg/kg that would be encountered by a 75 kg person consuming a 16-ounce soft drink.

Our group's interest in the metabolic consequences of sodium benzoate ingestion began with a study in which we used mass-spectrometry-based metabolite profiling of plasma to characterize the response to an oral glucose tolerance test (OGTT) [1]. The OGTT beverage contains 75 g of glucose, additives for taste and color, as well as the GRAS concentration of sodium benzoate as a preservative. The most significantly changed metabolite in that study was hippurate, originating from the sodium benzoate [1]. The significant rise in hippurate

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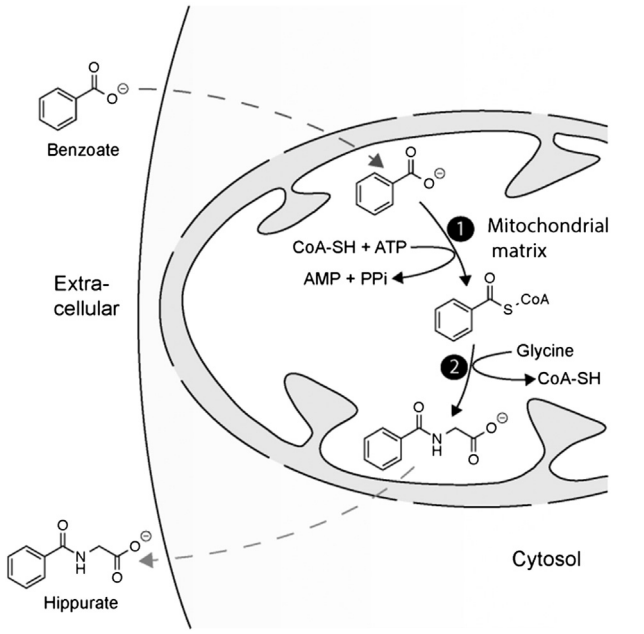

Fig. 1. Benzoate metabolism in mitochondria. Benzoate conversion to hippurate occurs within the mitochondrial matrix in two steps. Benzoate enters the mitochondria and is converted to benzoyl CoA (reaction 1) by an ATP-dependent acid:CoA ligase. Benzoyl CoA is subsequently converted to hippurate (reaction 2) by glycine N-acyltransferase, and then exits the mitochondria.

raised the question of what the broader metabolic impact of sodium benzoate is, and whether the sodium benzoate contained in the OGTT beverage influences the response to a glucose challenge. Given that the OGTT is used to diagnose diabetes mellitus, such an interaction would have important clinical implications. Even more concerning is the fact that if sodium benzoate significantly influences the response to a glucose challenge, frequent consumption of soft drinks and other foods containing benzoate could increase the risk of developing type 2 diabetes mellitus.

It is notable that several reports from animal and cell culture models have suggested that sodium benzoate and hippurate might have a significant impact on glucose homeostasis. For example, intravenous infusion of benzoic acid in sheep resulted in a rise of serum glucose, insulin, and glucagon without concurrent glucose administration [11], suggesting a combined secretagogic action on insulin and glucagon, as well as a potential influence on insulin sensitivity. Studies in patients with renal insufficiency demonstrated that hippurate impairs basal and insulin-stimulated glucose uptake into cells in culture [12,13]. The authors suggest that hippurate, which is renally-cleared, may accumulate in the setting of renal insufficiency and could therefore explain the altered glucose homeostasis observed in these patients. Finally, the package insert of the clinical formulation of intravenous sodium benzoate (which also contains sodium phenylacetate and dextrose) indicates that hyperglycemia was seen in seven percent of patients treated with intravenous high-dose benzoate [14].

Taken together, these data led us to formulate the novel hypothesis that sodium benzoate, at the GRAS dose, and its metabolite hippurate might impact insulin and glucagon secretion, as well as peripheral insulin action. However, no studies performed to date address the *in vivo* impact of a commonly encountered oral dose of sodium benzoate on glucose homeostasis in human subjects. The present study was designed to test the hypothesis that the GRAS dose of sodium benzoate has a significant impact on the response to a glucose challenge, and therefore might represent a potentially diabetogenic chronic environmental exposure. We report here the results of a randomized, cross-over trial conducted to assess the impact of acute oral administration of the GRAS dose of sodium benzoate, on its own and in combination with glucose, on glucose homeostasis and the plasma metabolite profile.

2. Results

In order to assess the metabolic impact of sodium benzoate, we recruited 14 overweight but otherwise healthy individuals into a randomized, controlled cross-over clinical trial. As summarized in Fig. 2, the individuals came in for two study visits at which they were given a water beverage followed by a 75-gram glucose solution. On one study day both beverages contained 0.1% sodium benzoate, and on the other study day they contained no sodium benzoate.

The characteristics of the recruited subjects are shown in Table 1. One subject had a greater than 2.5% change in weight between the study visits and one subject had both a greater than 8 week interval between study visits and a BMI slightly above the desired range of 25–30 kg/m^2. Given that these were not considered significant deviations from the study design and protocol, these individuals were included in the overall analysis.

Based on subject weights, the administered dose of 480 mg sodium benzoate corresponded to a per kg dose of 5.65 mg/kg/dose (range 4.5–7 mg/kg/dose), substantially lower than the doses used clinically for treatment of hyperammonemia and non-ketotic hyperglycinemia. The process measures for the study are displayed in Fig. 3, demonstrating

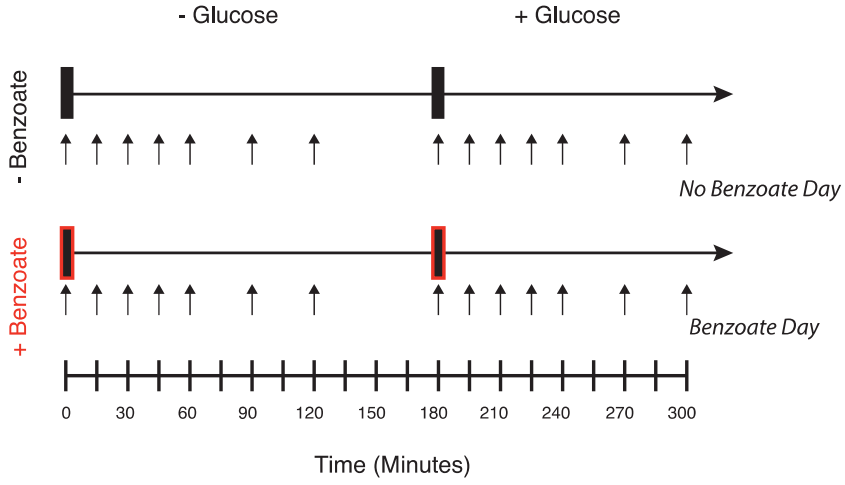

Fig. 2. Overview of study design. Two test beverages with or without 0.1% sodium benzoate were given on each of two test days at times 0 and 180 min. The second beverage always contained 75 g of glucose. Serial blood sampling was performed as indicated by the black arrows.

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