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Acetaminophen normalizes glucose homeostasis in mouse models for diabetes

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ABSTRACT

Loss of pancreatic beta cell insulin secretion is the most important element in the progression of type 1 and type 2 diabetes. Since oxidative stress is involved in the progressive loss of beta cell function, we evaluated the potential for the over-the-counter analgesic drug and antioxidant, acetaminophen (APAP), to intervene in the diabetogenic process. We used mouse models for type 1 diabetes (streptozotocin) and type 2 diabetes (high-fat diet) to examine the ability of APAP to intervene in the progression of diabetes. In C57BL/6J mice, streptozotocin caused a dosage dependent increase in fasting blood glucose (FBG), from 100 to >600 mg/dl. Daily APAP (20 mg/kg BW, gastric gavage), significantly prevented and partially reversed the increase in FBG levels produced by streptozotocin. After 10 weeks on a high-fat diet, mice developed fasting hyperinsulemia and impaired glucose tolerance compared to animals fed a control diet. APAP largely prevented these changes in insulin and glucose tolerance. Furthermore, APAP prevented most of the increase in body fat in mice fed the high-fat diet. One protective mechanism for APAP is suggested by studies using isolated liver mitochondria, where low micromolar concentrations abolished the production of reactive oxygen that might otherwise contribute to the destruction of pancreatic β-cells. These findings suggest that administration of APAP to mice, in a dosage used safely by humans, reduces the production of mitochondrial reactive oxygen and concomitantly prevents the development of type 1 and type 2 diabetes in established animal models.

1. Introduction

Obesity and diabetes are at epidemic proportions in American and Western populations [1]. Type 1 diabetes (previously termed juvenile onset diabetes) the most common chronic metabolic disease of childhood, results from an autoimmune-mediated loss of pancreatic islet β-cell mass. Type 2 diabetes (previously termed adult onset diabetes) usually

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Abbreviations: APAP, acetaminophen (N-(4-hydroxyphenyl)acetamide); FBG, fasting blood glucose; STZ, streptozotocin (1-methyl-1-nitroso-3-[2,4,5-trihydroxy-6-(hydroxymethyl)oxan-3-yl]-urea); T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus.

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occurs later in life and is typically preceded by obesity and associated metabolic abnormalities such as insulin resistance and dyslipidemia. Obesity is a primary risk factor for type 2 diabetes. Estimated U.S. costs for health care related to obesity are about $117 billion per year [2] and the years of life lost to obesity in the United States were estimated at 13–20 for males and 5–8 for females [3] In humans, a collection of conditions (elevated fasting blood insulin levels, glucose intolerance, an increase in abdominal fat, body mass index and blood lipid levels) is often termed metabolic syndrome [4,5]. The condition may be thought of as a cluster of risk factors for type 2 diabetes, or simply a prediabetic state. Weight loss may delay or prevent the onset of type 2 diabetes in obese individuals having impaired glucose tolerance, and even lessen the severity of insulin resistance [6,7]. Despite the growth of diabetes into one of the major causes of mortality, morbidity and health care expense in the U.S., and despite advances in treatments directed at correcting abnormal blood glucose, the development of preventive strategies have lagged.

Hyperglycemia is the major risk factor for development of diabetic microvascular diseases, including cardiomyopathy, nephropathy, retinopathy and peripheral neuropathy [8–12]. Hyperglycemia often develops after the onset of obesity, and intensifies through stages of insulin resistance and hypersecretion, with a slowly progressing loss of pancreatic islet β-cell mass and function. Eventually, the diminished β-cell mass cannot secrete sufficient insulin to support a compromised insulin-dependent glucose uptake in peripheral muscle, and blood sugar levels rise. Intensive treatment of diabetic patients, to lower blood glucose levels to near-normal values, significantly reduces microvascular complications of diabetics. However, in spite of aggressive drug therapy, insulin production in type 2 diabetes patients persistently declines [13]. Importantly, the fundamental feature of cellular oxidative stress and mitochondrial reactive oxygen production is central to the decline in β-cell mass [13–15], and hyperglycemic tissue damage [16–18]. These pathways include the formation of advanced glycation products, glucose metabolism through the polyol pathway, protein kinase C activation and the glucosamine pathway [19]. Such oxidative pathways that mediate hyperglycemic disease provide potential targets for therapeutic intervention.

Previous studies have shown that the widely used over-the-counter analgesic pharmaceutical, acetaminophen (APAP), has strong antioxidant properties in a number of biological systems [20–27]. Therefore, we examined the potential for APAP to intervene in the development of diabetes and obesity, using mouse models of type 1 diabetes and type 2 diabetes.

2. Materials and methods

2.1. Chemicals

All chemicals and reagents were obtained from Sigma–Aldrich Chemical Company (St. Louis, MO) as the highest available grades.

2.2. Animals and treatment

All experiments involving mice were conducted in accordance with the National Institutes of Health standards for care and use of experimental animals and the University of Cincinnati Institutional Animal Care and Use Committee. Female C57BL/6J mice were purchased from Jackson Laboratory (Bar Harbor, ME). Animals were group-housed, maintained on a 12 h light/dark cycle and had access to rodent chow and water *ad libitum*. Mice were matched by initial body weight, and assigned to groups. Mice were allowed *ad libitum* either a normal or a high-fat diet that was pelleted, semipurified and nutritionally complete. The normal diet (AIN-93M, Dyets Inc., Bethlehem, PA) contained 3 g of butter oil and 1 g of soybean oil/100 g diet, supplying 16.12 kJ/g diet, with 1.29 kJ of fat [28]. High-fat diet-fed mice received the AIN-93M diet containing 19 g butter oil and 1 g of soybean oil/100 g diet, supplying 19.34 kJ/g diet, with 7.74 kJ of fat. Both diets contained the same amount of protein, minerals and vitamins [29].

Where indicated, mice were treated with APAP by gavage once per day, between 10 am and 12 noon, at a dose (20 mg APAP/kg/d) that is well tolerated in humans and mice. Measurements were made, or mice were sacrificed, 20–24 h after the last APAP treatment. Streptozotocin (STZ) was dissolved in 10 mM citrate buffer (pH 5.0) and injected i.p. following an 8 h fast. Food was returned and the mice received 10% sucrose in the drinking water for 24 h, as described [30].

2.3. Glucose and glucose tolerance test

Glucose concentration was determined with a handheld glucometer (Ascensia Contour glucometer, Bayer) [29]. Bi-weekly samples of 5 μl blood were applied directly to the glucose strip from 8 h fasted mice to measure fasting levels of blood glucose. Glucose tolerance tests were performed after an 8 h fast. After initial blood glucose determinations, 1.5 mg D-glucose/g body weight was administered by i.p. injection, followed by glucose determinations at 20 min intervals for 120 min. Plasma insulin was measured using a radioimmunoassay employing guinea pig anti-insulin serum with high affinity for rodent insulin [31].

2.3.1. Histopathology

Histological examination was used to ensure that our dosing schedule with APAP was below the toxicity threshold to known target organs, such as liver, kidney and olfactory epithelium [32]. Tissues were examined after 4, 7 and 10 weeks of treatment with APAP, and compared to tissues taken from untreated control mice. Mice were euthanized by carbon dioxide asphyxiation, and target tissues were excised to 10% buffered formalin, then embedded in paraffin and sectioned. Sections (5 μm) for histopathological evaluation were stained with hematoxylin and eosin and evaluated, using light microscopy, by an observer blinded to animal treatment.

2.3.2. Metabolic parameters and body composition

Body weights were measured and food and water consumption were estimated twice weekly. In vivo oxygen consumption and CO_2 release were determined using metabolic chambers. Oxygen consumption was determined as gas