Monomeric cocoa catechins enhance β-cell function by increasing mitochondrial respiration

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Abstract

A hallmark of type 2 diabetes (T2D) is β-cell dysfunction and the eventual loss of functional β-cell mass. Therefore, mechanisms that improve or preserve β-cell function could be used to improve the quality of life of individuals with T2D. Studies have shown that monomeric, oligomeric and polymeric cocoa flavanols have different effects on obesity, insulin resistance and glucose tolerance. We hypothesized that these cocoa flavanols may have beneficial effects on β-cell function. INS-1 832/13-derived β-cells and primary rat islets cultured with a monomeric catechin-rich cocoa flavanol fraction demonstrated enhanced glucose-stimulated insulin secretion, while cells cultured with total cocoa extract and with oligomeric or polymeric procyanidin-rich fraction demonstrated no improvement. The increased glucose-stimulated insulin secretion in the presence of the monomeric catechin-rich fraction corresponded with enhanced mitochondrial respiration, suggesting improvements in β-cell fuel utilization. Mitochondrial complex III, IV and V components are up-regulated after culture with the monomer-rich fraction, corresponding with increased cellular ATP production. The monomer-rich fraction improved cellular redox state and increased glutathione concentration, which corresponds with nuclear factor, erythroid 2 like 2 (Nrf2) nuclear localization and expression of Nrf2 target genes including nuclear respiratory factor 1 (Nrf1) and GA binding protein transcription factor alpha subunit (GABPA), essential genes for increasing mitochondrial function. We propose a model by which monomeric cocoa catechins improve the cellular redox state, resulting in Nrf2 nuclear migration and up-regulation of genes critical for mitochondrial respiration, glucose-stimulated insulin secretion and ultimately improved β-cell function. These results suggest a mechanism by which monomeric cocoa catechins exert their effects as an effective complementary strategy to benefit T2D patients.

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1. Introduction

The incidence of type 2 diabetes (T2D) is increasing at an alarming rate. There are an estimated 415 million people worldwide that are diabetic, with current projections of 642 million people being diabetic by 2040, with the vast majority suffering from T2D [1]. T2D is characterized by hyperglycemia and hyperlipidemia due to muscle, adipose and liver insulin resistance [2,3]. In addition, studies have begun to demonstrate that decreased functional β-cell mass is an early and essential step in T2D disease progression [4].

The primary purpose of the β-cell is to maintain normoglycemia through glucose-stimulated insulin secretion (GSIS). The β-cell dysfunction observed in T2D results in decreased and poorly controlled insulin secretion and, ultimately, β-cell death [4]. Mechanisms that increase functional β-cell mass could, therefore, be leveraged as a treatment for T2D.

Recent studies have shown that diets supplemented with cocoa or flavanols present in cocoa have potentially beneficial effects for the T2D patient [5]. Some of these studies have suggested that these effects may include increased cellular antioxidant abilities [6,7]. Given the fact that β-cells are particularly sensitive to oxidative stress, flavanol compounds found in cocoa may have direct therapeutic applications for the control and prevention of T2D [8].
Cocoa contains a complex profile of monomeric flavanols (catechins such as epicatechin) and their oligomeric and polymeric forms (procyanidins) (Fig. 1). Using fractionation methods, our group has begun to differentiate the effects of these distinct flavanol fractions. We have previously shown that these fractions have different whole-body effects in regard to diet-induced T2D. These findings demonstrated that oligomeric cocoa procyanidins have the greatest effect in preventing high-fat-feeding-induced weight gain while maintaining normal fasting glucose, insulin levels, glucose tolerance and insulin sensitivity [9]. However, these are whole-body effects, and studies are needed to describe the effect of various cocoa flavanols fractions on various tissues affected by T2D. We recently demonstrated differential impacts of these fractions on insulin signaling and glucose utilization in skeletal muscle cells [10]. However, no data exist on how these fractions may affect pancreatic β-cell function, which is critical for preventing or ameliorating T2D.

Previous studies suggest that cocoa flavanols protect and/or improve β-cell function. These effects appear to include enhanced survival, insulin secretion and proliferation. In vitro, cocoa flavanols protect against oxidative stress in INS-1 cells and increase insulin secretion from rat islets [11]. In Zucker diabetic fatty rats, dietary cocoa prevents β-cell apoptosis by reducing oxidative stress and also increases small islet size and maintenance of total islet mass, suggesting induction of β-cell proliferation [7]. In ob/ob and normal rats, cocoa flavanol treatment enhances insulin secretion [6]. In a nonobese type 1 diabetic model, application of cocoa flavanols increases β-cell mass [12]. Additionally, cocoa flavanol treatment enhances DNA replication and regeneration of β-cells in rats [13–15]. Finally, in humans, cocoa flavanols enhance postprandial insulin secretion [16]. Despite these promising results, the molecular mechanisms by which these improvements occur are yet to be elucidated.

The transcription factor nuclear factor, erythroid 2 like 2 (Nrf2, also known as NFE2L2) controls the cellular response to oxidative stress [17]. Under normal conditions, Nrf2 is bound in the cytosol by the Kelch-like ECH-associated protein 1 (Keap1) and targeted for proteolytic degradation, thus suppressing Nrf2-mediated transcriptional activity [18]. Under oxidative stress conditions, however, Keap1 cysteine residues are oxidized, resulting in Nrf2 release and nuclear translocation [19]. Upon nuclear translocation, Nrf2 induces expression of genes whose promoters contain antioxidant response elements (ARE) [20]. Many of these Nrf2-regulated genes are involved in processes to quench free radicals and ultimately up-regulate the cell’s ability to decrease oxidative stress. While direct Nrf2 targets involved in antioxidant response have been defined for some time, recent studies have demonstrated that Nrf2 also enhances expression of genes that regulate mitochondrial function [21].

The transcription factors nuclear respiratory factor 1 (Nrf1) and GA binding protein transcription factor alpha subunit (GABPA, also known as nuclear respiratory factor 2) are essential for mitochondrial function and biogenesis [22]. These transcription factors directly control expression of genes involved in the electron transport chain, as well as enhance expression of genes such as TFAM that drive mitochondrial production [22,23]. Cellular response to events that require increased mitochondrial function are dependent on the function of these transcription factors. Given that GSIS is intimately connected to mitochondrial respiration, these transcription factors are especially important for β-cell function [24]. Therefore, the goal of this study was to determine the effect of flavanol fractions on GSIS and to define the mechanism by which these compounds exert their effects.

2. Materials and methods

2.1. Animal husbandry and islet isolation

Wistar rat breeding pairs were purchased from Harlan and maintained on standard chow diet (Teklad 7001; Harlan). Pups were weaned at 21 days. Male rats were allowed to feed ad libitum and were maintained on a 12-h light–dark cycle. Rats were age- and weight-matched for all islet experiments. Pancreatic islets were isolated from 5-week-old male rats as previously described [25–28]. Primary rat islets were cultured in RPMI 1640 and supplemented with 10% fetal bovine serum, 1% Fungizone antymycotic (Life Technologies) and 1% HEPES. Islet medium was changed every 24 h. All animal studies were approved and performed in accordance with Brigham Young University’s Institutional Animal Care and Use Committee guidelines (protocol #16-0902).

2.2. Cocoa extraction and fractionation

We selected fractionation in order to examine the activities of groups of compounds together, as opposed to individually, due to the scarcity of commercial standards for procyanidins larger than tetramers. A flavanol-rich cocoa extract was produced from commercially available cocoa powder and fractionated into monomeric catechin-rich, oligomeric procyanidin-rich and polymeric procyanidin-rich fractions. The production and composition of these fractions have been described previously [9]. Detailed methodologies are presented in Supplementary Information. Characterization and enrichment levels of monomeric catechins and procyanidins in the extract and individual fractions are presented in Supplementary Material (Table S1, Figs. S1–S2).