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High-molecular-weight cocoa procyanidins possess enhanced insulin-enhancing and insulin mimetic activities in human primary skeletal muscle cells compared to smaller procyanidins

Suzanne M. Bowser[a], William T. Moore[a], Ryan P. McMillan[a,b], Melanie R. Dorenkott[c,1], Katheryn M. Goodrich[c,2], Liyun Ye[c,3], Sean F. O'Keefe[c], Matthew W. Hulver[a,b,*], Andrew P. Neilson[c,**]

[a]Department of Human Nutrition, Foods and Exercise, Virginia Tech, Blacksburg, VA, USA
[b]Virginia Tech Metabolic Phenotyping Core Facility, Virginia Tech, Blacksburg, VA, USA
[c]Department of Food Science and Technology, Virginia Tech, Blacksburg, VA, USA

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Abstract

Dysregulation of glucose metabolism is a primary hallmark of metabolic disease (*i.e.*, diabetes, obesity, *etc.*). Complementary nonpharmaceutical strategies are needed to prevent and/or ameliorate dysregulation of glucose metabolism and prevent progression from normoglycemia to prediabetes and type 2 diabetes across the lifespan. Cocoa compounds, particularly the procyanidins, have shown promise for improving insulin sensitivity and blood glucose homeostasis. However, the molecular mechanisms by which cocoa procyanidins exert these functions remain poorly understood. Furthermore, cocoa procyanidins exhibit size diversity, and evidence suggests that procyanidin bioactivity and size may be related. Here, we show that a procyanidin-rich cocoa extract elicits an antidiabetic effect by stimulating glycogen synthesis and glucose uptake, independent of insulin. Cocoa procyanidins did not appear to act *via* stimulation of AMPK or CaMKII activities. Additionally, in the presence of insulin, glycogen synthesis and AKT phosphorylation were affected. These mechanisms of action are most pronounced in response to oligomeric and polymeric procyanidins. These results demonstrate (1) specific mechanisms by which cocoa procyanidins improve glucose utilization in skeletal muscle and (2) that larger procyanidins appear to possess enhanced activities. These mechanistic insights suggest specific strategies and biological contexts that may be exploited to maximize the antidiabetic benefits of cocoa procyanidins.

Keywords: Glucose; Glycogen synthesis; GLUT4; AKT; CaMKII; AMPK

1. Introduction

Recently, there has been interest in the potential for cocoa flavanols to prevent and/or ameliorate obesity, prediabetes and type 2 diabetes mellitus (T2DM) [1]. Human studies incorporating cocoa and cocoa products have shown promising effects, including improved postprandial insulin secretion [2], insulin sensitivity [3–5], blood glucose control [6] and increased skeletal muscle mitochondrial biogenesis [7]. In animals, dietary supplementation with cocoa or cocoa flavanols significantly reduces weight and fat gain, obesity-associated inflammation, fasting blood glucose levels and glucose intolerance in various animal models of obesity and/or T2DM [8–17]. Flavanols are thought to exert these antiobesity and antidiabetic activities by inhibiting digestive enzymes [18], enhancing β-cell function [19,20], inhibiting metabolic endotoxemia and associated metabolic derangements [15] and improving insulin sensitivity in liver [21] and skeletal muscle [1,22–27].

Flavanols are a diverse class of compounds comprised of monomers [(±)-catechin (C), (−)-epicatechin (EC), (−)-epicatechin gallate, *etc.*], as well as oligomers and polymers known as the procyanidins (PCs) (representative flavanols in cocoa are shown in Fig. 1). Flavanol compounds are characterized by their degree of polymerization (DP) [28], which refers to the number of monomer residues linked together. Monomers have DP=1, oligomers have DP=2–8 or 2–10 (definitions vary) and polymers have DP>8–10. Foods or extracts containing flavanols can be characterized by their mean DP (mDP), which is the average DP of all flavanols present [29,30]. PCs can be further characterized as "A-type" or "B-type," based on the nature of the bonds connecting the monomer residues (A-type and B-type PCs

* Correspondence to: M. W. Hulver, PhD, Department of Human Nutrition, Foods and Exercise and The Virginia Tech Metabolic Phenotyping Core Facility, 338 Wallace Hall, 295 West Campus Dr., Blacksburg, VA 24061, USA. Tel.: +1 540 231 7354; fax: +1 540 231 3916.
** Correspondence to: A. P. Neilson, PhD, Department of Food Science and Technology, Virginia Tech, 1981 Kraft Dr., Blacksburg, VA 24061, USA. Tel.: +1 540 231 8391; fax: +1 540 231 9293.
E-mail addresses: hulvermw@vt.edu (M.W. Hulver), andrewn@vt.edu (A.P. Neilson).
[1] Current affiliations: University Hospitals, Cleveland, OH.
[2] Current affiliations: DuPont Crop Protection, Newark, DE.
[3] Current affiliations: SPF North America Inc., Greenville, SC.

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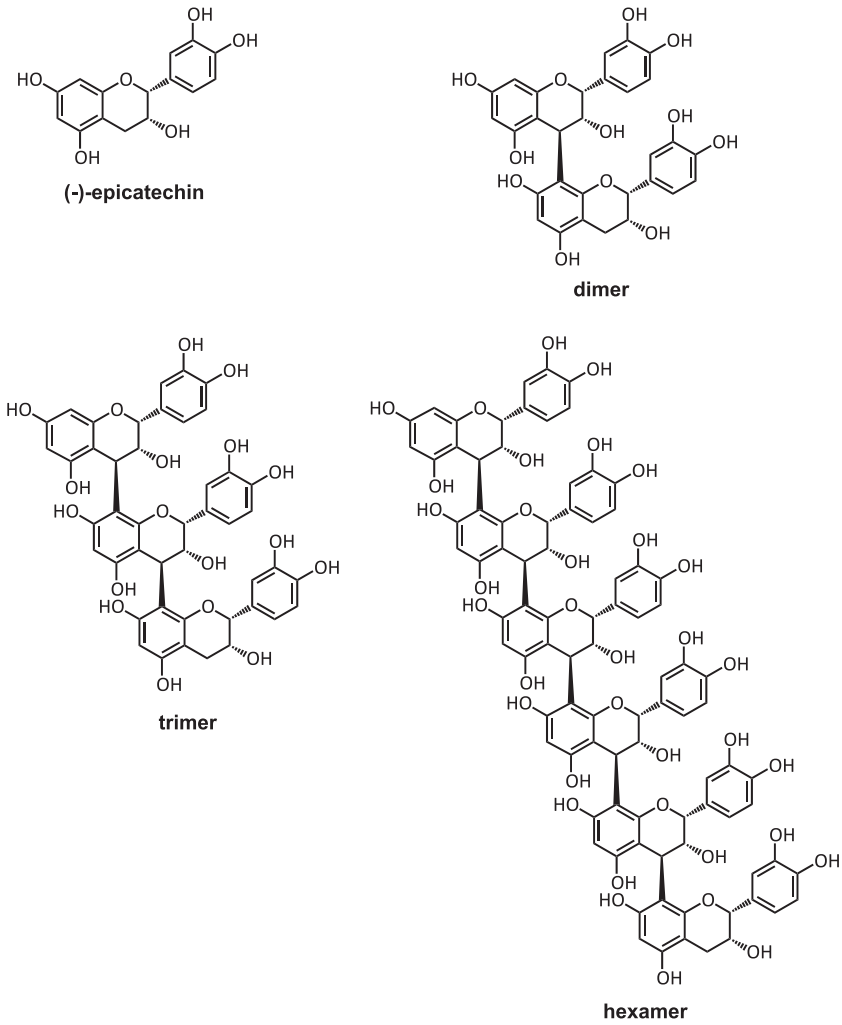

Fig. 1. Structures of (−)-epicatechin and a representative (−)-epicatechin dimer, trimer, and hexamer linked by 4β-8 linkages.

have monomer residues linked by 1 and 2 bonds, respectively, connecting distinct positions). Cocoa and cocoa products are among the most flavanol-rich foods and display a wide range of B-type PCs with varying DPs [31–33]. The main flavanol monomer in cocoa is (−)-EC, with lower levels of (−)-C and (+)-C [34–36]. Cocoa is also rich in in B-type PCs up to DP 10–12 [28,37,38].

Due to the structural complexity of cocoa flavanols, it is important to determine whether specific individual flavanols or groups of flavanols are primarily responsible for the observed activity and, if so, identify these compounds and the mechanisms by which they act. Furthermore, different flavanols or groups of flavanols may act by distinct, complementary mechanisms. Recently, we demonstrated that oligomeric cocoa PCs possess enhanced antidiabetic and antiobesity efficacy compared to monomeric and polymeric PCs in a mouse model of prediabetes [39]. The mechanisms by which oligomers preferentially exert these effects are of great interest for enhancing cocoa bioactivities. These mechanisms may include inhibition of carbohydrate digestion, which has been shown to be DP-dependent [18,40]. Previous work has also shown that a tetrameric cocoa PC (cinnamtannin A_2) preferentially stimulates glucagon-like peptide-1 and insulin secretion compared to monomeric, dimeric and trimeric PCs [41]. Another mechanism of interest is the modulation of insulin signaling and sensitivity in skeletal muscle. Cinnamtannin A_2 enhances the activities of various components of the insulin signaling pathway in skeletal muscle compared to PCs with DP≤3 [41]. Conversely, PCs with DP≤3 were found to be more effective at stimulating AMPK phosphorylation and GLUT4 translocation in L6 myotubes compared to higher MW PCs (DP≥4) [27,42]. Additionally, PC oligomers from grape seeds appear to stimulate insulin signaling pathways independent of insulin [24]. Therefore, PCs appear to improve glucose utilization in skeletal muscle in a DP-dependent manner, but the mechanisms by which this occurs remain poorly understood. Our lab and others have demonstrated enhanced antidiabetic and antiobesity activities in response to PCs with higher DPs [9,23,24,39,41]. These studies demonstrated the ability of cocoa flavanols to stimulate insulin-signaling pathways, independent of insulin (*i.e.*, insulin mimetic activity). However, we do not know the specific mechanisms whereby this occurs, as previous studies have demonstrated up-regulation of activity, but have not probed mechanisms. Furthermore, the impact of larger PCs on these processes remains largely unknown.

The objective of this study was therefore to compare the impacts of a PC-rich cocoa extract (CE) and cocoa PC fractions with distinct mDPs on insulin signaling and glucose metabolism in skeletal muscle, and interrogate the mechanisms by which these effects occur. Based on our previous results [39], we hypothesized that cocoa PCs would increase insulin-stimulated glucose metabolism, with oligomeric PCs being the most effective fraction compared to monomers and polymers.