Hypoglycemic property of cocoa products: potential underlying mechanisms

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ABSTRACT
Cocoa powder and chocolate is most abundant flavonoids-plant product. Type 2 diabetes mellitus (T2DM) is health and epidemically serious metabolic disorder. The treatment and moreover preventing of T2DM is therapeutic target worldwide. This review focuses on antidiabetic mechanisms of cocoa products. Particularly, intensifying insulin sensitivity, which is a superior pathway for chronic glucose level control, and increasing insulin release for acute glucose level control. Accordingly, the hypoglycemic effect results in elevate synthesis and bioavailability of nitric oxide (NO). Subsequently, improve blood flow and capillary recruitment.

Key Words: Cocoa flavonoids, chocolate, nitric oxide, diabetes mellitus, hypoglycemic.

INTRODUCTION
Insulin resistance is a physiological conditions in which normally insulin-sensitive cells show weak response to insulin (Clark et al., 2003) such as impaired glucose tolerance (IGT) (Association, 2010), in which reveal sustained hyperglycemia (Baron et al., 1996, Kim et al., 2006), with high incidence of type 2 diabetes mellitus (T2DM) occurrence (Osei et al., 2004). T2DM is one of the most global health burden disorder and epidemically growing (Whiting et al., 2011). Management and protection of T2DM patients from sudden and persistent increasing of glucose level in the blood is an essential aim of treatment (Gennuth, 2003).

Natural flavonoids from plant source are a novel agent candidate for diabetes mellitus (DM) therapy (Hanhineva et al., 2010). Flavonoids are polyphenolic compound abundantly found and detected in vegetables and fruits (Scalbert and Williamson, 2000). The regular intakes of polyphenolic rich diets or beverages bypass the deterioration prognosis of many disorders such as atherosclerosis and DM (Kosinska and Andlauer, 2012). Polyphenolic compounds have diverse health benefits for instance antioxidant, cardioprotective and anticarcigenic (Jaganath and Crozier, 2009). The production of raw cocoa powder or chocolate required many processing steps, namely, fermentation, roasting, grinding and alkalizing, subsequently influence the polyphenolic contents of final products (Wollgast and Anklam, 2000). Even so Cocoa powder and chocolate have remained one of the richest flavonoids plant products (Sanbongi et al., 1998). Cocoa and chocolate polyphenols composed mostly of monomers (catechin and epicatechin) and oligomers (procyanidins) (Bravo, 1998; Tomás-Barberán et al., 2007). Furthermore, methylxanthines, which are caffeine, theobromines, and theophylline, have been identified as well (Kelm et al., 2006). This review points out the cocoa powder and chocolate potential hypoglycemic mechanisms, which are enhanced insulin release and sensitivity, antioxidant and angiotensin converting enzyme (ACE) inhibitor. Interestingly, boosting insulin sensitivity is mostly a predominant mechanism for chronic glucose level control through stimulation of insulin signaling mediators (Klover and Mooney, 2004; Cao et al., 2007; Cordero-Herrera et al., 2013; Zhang et al., 2013; Cordero-Herrera et al., 2014). Moreover, the augmenting of insulin release is likely to be acute glucose level control mechanism (Rabinowitz et al., 1966; Brand-Miller et al., 2003; Mhd Jalil et al., 2009; Sarmadi et al., 2012; Martin et al., 2014). The evoked sensitivity and release of insulin result in amelioration of vasodilatation and/or capillary recruitment in which led to increase muscle blood flow (Steinberg et al., 1994; Clark et al., 2003) due to either direct vasodilatation effect of insulin (Schultz et al., 1977; Chen and Messina, 1996) or insulin signaling mediators which led to boost nitric oxide NO production (Kim et al., 2001).

HYPOGLYCEMIC EFFECT
Abbe et al. investigation arises short-term glucose control (at 60 and 90 min postprandial) of obese-diabetic (ob-db) rats supplemented with cocoa extract contains polyphenols, 3.55mg caffeine and 2.22mg theobromines /g cocoa extract (Mhd Jalil et al., 2009). Consistent with this observation, cocoa supplementation boosted insulin release in blood of healthy adults (Brand-Miller et al., 2003; Martin et al., 2014). Noticeably, insulin has crucial hemodynamic impact, which affect muscle metabolism (Vincent et al., 2004). It has direct vasodilator action through insulin receptors (IR) on vascular cells (Schultz et al., 1977; Chen and Messina, 1996). In addition, the vasodilatation implicated nitric oxide (NO) synthesis by endothelial cells (Schultz et al., 1977) through stimulation of signaling cascade that led to activation of endothelial nitric oxide synthase (eNOS) by IRS-1, phosphatidylinositol 3-kinase (PI3K) and protein kinase B (AKT) (Kim et al., 2001). Furthermore, insulin modulates capillary recruitment in vivo (Baron et al., 1993; Vincent et al., 2004) through direct dilatation effect on blood vessels bed by enhancing NO generation from endothelial cells (Ziai et al., 2005) or from skeletal muscles (Grassi et al., 2008). Eventually, insulin-mediated elevation in muscle blood flow and/or capillary recruitment led to improve glucose uptake in healthy adult (Clark et al., 2003), and inversely proportional to the blood pressure (Ruzaidi et al., 2008).
However, the amelioration of insulin plasma level is not only the sole mechanism of polyphenolic antidiabetic effect in T2DM (Lee et al., 2005), glucose-intolerant adult (Suzuki et al., 1995), and STZ-induced diabetic rats (Suzuki et al., 1995). Moreover, the cocoa supplementation shows long-term glucose control in both (ob-db) mice (Vincent et al., 2002), and streptozotocin (STZ)-induced diabetic rats (Shankar et al., 2000; Iwashita et al., 2001). Interestingly, medicines that ameliorate vasodilation, such as angiotensin-converting enzyme inhibitors (Amin et al., 2004; Tomaru et al., 2007), α-blockers (Fogari et al., 1998; Ruzaidi et al., 2005), are also attenuate the insulin-resistance. So that, the hypoglycemic effect of cocoa extract presumed to be owed to stimulate insulin release (Suzuki et al., 1995), and/or sensitivity (Suzuki et al., 1995; Andersson and Lithell, 1996). Subsequently, cocoa-polyphenols may have not restricted enhancement effect on insulin release only.

However, theobromines display no hemodynamic and electrophysiological impacts on healthy adults (Suzuki et al., 1992). While, caffeine intakes led to critical suppression of insulin sensitivity in sedentary subjects (Baron et al., 1999), and T2DM patients (Greer et al., 2001). Hence, caffeine has partly implicated in alleviating glucose uptake (Mhd Jalil et al., 2009).

The one more hypoglycemic potential mechanism of polyphenols is their impact on liver, muscles and intestine. The liver is one of the substantial organs, which regulate the blood glucose concentration within the normal ratio by controlling both gluconeogenesis and glycolysis for glucose supplementation during hypoglycemic condition and elevate glucose disposal from blood circulation to boost glycogen synthesis through hyperglycemia (Klover and Mooney, 2004). Whilst, diminishing in insulin’s ability to elevate glycogen synthesis in the liver is one of hepatic insulin resistance characteristic features (Klover and Mooney, 2004).

Cocoa polyphenolic extract (CPE), and essential cocoa flavanol, (-)-epicatechin (EC), promote insulin sensitivity of hepatic cells (HepG2), by the suppression of alleviating levels and tyrosine-phosphorylated of insulin receptor (IR), insulin receptor substrate-1 (IRS-1) and insulin receptor substrate-2 (IRS-2) induced by high glucose concentration (Cordero-Herrera et al., 2014), which have a critical role in hepatic insulin resistance progression (Nakajima et al., 2000; Klover and Mooney, 2004). Furthermore, stimulation of IR, IRS-1and IRS-2 led to activation of AKT (PI3K)/PKB pathway (Klover and Mooney, 2004). CPE, EC and other naturally occurring polyphenols activate hepatic glycogen synthase (GS) by boosting the expression levels of PI3K/AKT and GSK3 in HepG2 cell line (Mhd Jalil et al., 2009) and liver of insulin-resistant rats (Brand-Miller et al., 2003; Martin et al., 2014). CPE and EC elevated 5-AMP-activated protein kinase (AMPK) phosphorylated level in induced insulin resistance HepG2 (Iwashita et al., 2001; Mhd Jalil et al., 2009) and cocoa liquor normalized P-AMPK in the liver of insulin resistance mice (Yamashita et al., 2012). However, AMPK alleviated high glucose-induced insulin resistance hepatic cells (Zang et al., 2004). The concomitant scarcity of GLUT-2 levels with insulin resistance (Nakajima et al., 2000) can be reverted in HepG2 cells by treatment with CPE and EC (Cordero-Herrera et al., 2014). The increasing of gluconeogenesis owing to rise of phosphoenolpyruvate carboxykinase (PEPCK) levels (Klover and Mooney, 2004), which associated with induced insulin resistance of both HepG2 cell line and mouse liver, have been prevented by using of CPE, EC (Cordero-Herrera et al., 2013; Cordero-Herrera et al., 2014) and other natural polyphenolic compounds (Collins et al., 2007a; Pu et al., 2012).

From the above results, we can conclude that CPE and EC reduced glucose synthesis and maintain or enhance glycogen production in induced insulin resistance liver or hepatic cells through improvement of AKT, AMPK, and GSK3, p-IRS-1, p-IRS-2 and GLUT-2 levels. Consequently, CPE and EC ameliorated liver insulin sensitivity and inhibited deterioration of insulin. Interestingly, the using of N-acetylcysteine as antioxidant treatment led to repress synthesis of anti-inflammatory mediator rather than bolstering of insulin sensitivity (Setshedi et al., 2011).

Moreover, The treatment of L8 muscle cells with the Canna indicia root extract at doses of 0.1-0.5 mg/ml, which contains flavonoids and catecholest, displayed rising in the amount of glucose transporter isoforms 1 (GLUT1) and 4 (GLUT4) at the muscle cell surface and boosted GLUT1 protein synthesis. In addition, it stimulates phosphatidylinositol 3-kinase (PI3K) as well (Purintrapibhan et al., 2006).

Whilst, dietary polyphenols suppress the intestinal cell line glucose uptake (Johnston et al., 2005) by the repression of sodium-dependent glucose transporter (SGLT1) (Kobayashi et al., 2000), which is crucial for intestinal glucose active transport (Wright and Turk, 2004). Likewise, cocoa acetone-dry powder (AcDP) autolysis at PH 3.5 displayed higher α-amylase inhibition effect (Sarmadi et al., 2012). Accordingly, it sustained the carbohydrate degradation and decreased glucose absorption. Eventually, it reduced the postprandial plasma glucose rise (De Fronzo et al., 2004). As well as, in vitro study the autolysate yielded at PH 3.5 showed a high insulinitropic effect (Sarmadi et al., 2012) that led to increase insulin secretion, which is responsible for the hypoglycemic effect following protein ingestion (Rabinowitz et al., 1966). The yield of (AcDP) autolysis at PH 3.5 is mostly oligopeptides with hydrophobic amino acid residue (Amin et al., 2002). The comprising amino acids in peptides are not only responsible for their actions, but their structure and sequence also (Chen et al., 1998).

From all together previous observations, it can be assumed that the likely hypoglycemic effects of cocoa and chocolate is resulting from short-term glucose lowering action, where insulin secretion is increasing (Rabinowitz et al., 1966; Brand-Miller et al., 2003; De Fronzo et al., 2004; Mhd Jalil et al., 2009; Sarmadi et al., 2012; Martin et al., 2014), and long-term glucose level decreasing effect, which included boosting of insulin sensitivity (Suzuki et al., 1995; Andersson and Lithell, 1996; Klover and Mooney, 2004; Purintrapibhan et al., 2006; Cao et al., 2007; Yamashita et al., 2012; Zhang et al., 2013; Cordero-Herrera et al., 2014) through immediate excitation of one or more of insulin signaling mediators cascade namely, AKT, AMPK, GSK3, p-IRS-1, p-IRS-2 and GLUT-2 in muscle cells and liver cells. Whilst, polyphenols decrease SGLT1 levels in intestine cells to alleviate glucose absorption and subsequently decrease the glucose concentration in blood (Kobayashi et al., 2000; Johnston et al., 2005).

**ANTIOXIDANT EFFECT**

Vascular endothelial cells with High glucose levels show reactive oxygen species (ROS) production (Du et al., 2001). Consequently, aggravate oxidative stress condition (Kopprasch et al., 2002), where ROS interact with NO to yield peroxyxinate (Lee et al., 2003). Consequently, led to alleviate NO bioavailability (Beckman et al., 2001), and disturbs the balance of vasodilation and vasocostriction factors (Beckman et al., 2001; Grassi et al., 2009). Therefore,
ROS inactivate endothelial NO and decrease it is vasodilation effect in diabetic adults (Kim et al., 2006). Whilst, antioxidant impact of vitamin C infusion normalizes vasodilation of type1 and type2 diabetic patients (Ting et al., 1996; Timimi et al., 1998). Interestingly, insulin infusion boost glucose uptake of muscle cells through improving of blood flow and capillary bed recruitment of skeletal muscles (SkM) via amelioration NO bioavailability (Baron et al., 1996; Clark et al., 2003). Furthermore, insulin treatment usage in enhancing PDK and/or AKT in human umbilical vein endothelial cells (Zeng et al., 2000), for optimization of glucose metabolism and blood flow (Muniyappa et al., 2007). Hence, insulin sensitivity partly depends on NO bioavailability in vascular endothelial cells (Konopatskaya et al., 2003), particularly in subjects with IGT (Hirai et al., 2000). Subsequently, hypoglycemic agents who increase the insulin sensitivity such as thiazolidinediones are likely to reduce the blood pressure (Nolan et al., 1994; Potenza et al., 2006) and elevate glutathione level in SKM of T2DM (Lazzich et al., 2012). However, epicatechin-rich cocoa (ERC) treatment lead to retrieve the normal level of glutathione in SKM of T2DM and heart failure (HF) patients (Ramirez-Sanchez et al., 2013). It has been demonstrated that flavanols boost the bioavailability of NO in endothelial cells through either it is insulin metabolic like effects, whereby interfering with vasodilation action of insulin (Schaer et al., 2006; Kim et al., 2007; Steffen et al., 2008), and/or in partly, it is antioxidant effects (Fischer et al., 2003; Collins et al., 2007b); this may be owe to protect of NO from destruction via ROS actions (Grassi et al., 2009), stimulate the innate antioxidant enzymes superoxide dismutase (SOD), whereby superoxide anion converted to oxygen molecule and hydrogen peroxide in vivo (Malstrom et al., 1975) and catalase, whereby hydrogen peroxide converted to water and molecular oxygen (Basan et al., 2009), in thymus of young rats supplemented by cocoa-enriched diet (Ramiro-Puig et al., 2007).The stimulation of antioxidant enzymes is likely through enhancement of SIRT3 (sirtuin), FOXO1 and PGC1α nuclear translocation and increasing their activating forms in skeletal muscles of T2DM and HF patients supplemented with ERC (Karim et al., 2000). Furthermore, flavanols has ameliorating effects to endothelial nitric oxide synthesis (eNOS) in blood vessel (Karim et al., 2000; Xu et al., 2004; Wallerath et al., 2005). Thus, healthy adults consumption of flavanol-rich cocoa led to increase bioavailability of endothelial NO (Engler et al., 2004; Fisher and Hollenberg, 2006), which is promoting for flow-mediate dilation (FMD) in elderly human (Fisher and Hollenberg, 2006), healthy subject (Fisher et al., 2003), and conduit arteries and microcirculation, where bypassed by using L-Nn-mono-methyl-arginine as a NOS inhibitor (Schaer et al., 2006). In addition, alleviated endothelin (ET-1) levels may be one of flavanols antioxidant mechanisms (Corder et al., 2001; Corder et al., 2004), which ameliorate FMD in healthy volunteers treated with flavanol-rich dark chocolate (Grassi et al., 2012).

**RENIN-ANGIOTENSIN SYSTEM MODULATION EFFECT**

Angiotensin I converting enzyme (ACE) is a glycoprotein peptidylpeptide hydrolase, whose play substantial role in controlling renin-angiotensin system, whereby angiotensin-I converted to angiotensin-II (Corvol et al., 1995), is potent vasoconstrictor (Dzau, 2001). Interestingly, ACE inhibitors and Angiotensin receptor blockers significantly diminished peripheral vascular resistance and ameliorate vascular recruitment concomitant with improvement of insulin resistance (Koh et al., 2005; Koh et al., 2007) and supported antioxidant system of human body (de Cavanagh et al., 2000). It was demonstrated that pure flavanols and procyanidins inhibit the ACE action (Actis-Goretta et al., 2003). Moreover, chocolate extracts ACE quenching effects relay on flavanols content and the number of epicatechin units forming the procyanidin (Actis-Goretta et al., 2006) and epicatechin tetramer possess the potent inhibition impact on ACE (Ottaviani et al., 2006). In addition, the enhancing insulin resistance effect of ACE inhibitors is likely through stimulation of bradykinin-NO system which results in boosting of GLUT4 translocation and subsequently elevates glucose uptake by peripheral tissues particularly in skeletal muscle of type 2 diabetic mice treated with temocapril (Shiuichi et al., 2002).

**CONCLUSION**

DM is chronic, bad prognosis and widely spread disease nowadays. Considerable synthetic antidiabetic drugs are available. However, they are not safe, cheap and convenient like natural one. The polyphenolic rich plants are emerging hypoglycemic naturally occurring agent. Particularly, cocoa powder and chocolate rich with flavonoids owe to their prospective valuable beneficial application in medicine. Therefore, it is insisted to know and elucidate their antidiabetic mechanism for ultimate employing of cocoa products in manufacturing of pharmaceuticals and nutraceuticals.

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