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Type 2 diabetes diminishes the benefits of dietary antioxidants: Evidence from the different free radical scavenging potential

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ABSTRACT

The development of food fortified with polyphenols and polyphenol-rich foods represents a novel approach for preventing or managing type 2 diabetes. Herein, taking advantage of several radical scavenging, the impact of plasma proteins in diabetes on the benefits of dietary polyphenols was investigated. It illustrated that plasma proteins masked the dietary polyphenols, thus reducing their radical scavenging potential. The plasma proteins from type 2 diabetics bind and protect (i.e., mask) the polyphenol antioxidants less effectively than the non-glycosylated ones in healthy blood do. In the blood of diabetics the less-protected (non-masked) antioxidants react with free radicals before being delivered to the tissues that need them. We should pay more attention to *in vivo* benefits of dietary polyphenols for type 2 diabetics.

1. Introduction

Diabetes mellitus is one of the most significant public health problems in the world. The number of diabetics is expected to reach 438 million by 2030 (approximately 7.5% of the adult population) (Wild, Roglic, Green, Sicree, & King, 2004). Recently, the WHO (2013) reported that there are about 347 million people worldwide have diabetes. Moreover, the majority of this increase was reported in developing countries (South America, China, and India), which are undergoing westernisation. For example, more than 100 million Chinese developed to type 2 diabetes in 2012. Diabetes is characterised as hyperglycemia with a high level of glucose (>6.10 mM), which can react with plasma proteins through a non-enzymatic process to form glycated hemoglobin and glycated albumin (Godzien, García-Martínez, Martinez-Alcazar, Ruperez, & Barbas, 2013).

These non-enzymatic modifications via the Maillard reaction have far-reaching effects on the metabolism and regulation, and may be responsible for increased infection and cancer rates within diabetics (Bierhaus, Hofmann, Ziegler, & Nawroth, 1998). Recently, Zwang, Gormally, Johal, and Sazinsky (2012) hypothesised that the glycation of serum proteins may improve the available free iron pool for bacteria in blood serum and weaken our innate immunity. Using fluorescence spectroscopy we have showed that the increasing glycation of plasma proteins in type 2 diabetics' blood can reduce their binding capability for dietary polyphenols (Xie, Xiao, Kai, & Chen, 2012).

Natural polyphenols especially flavonoids, phenolic acids and stilbenes are the most important antioxidants in human diets and have attracted great interests since the 1990s due to growing evidences of their beneficial effect on human health (Deng et al., 2013; Shen & Chang, 2013; Xiao & Högger, 2014; Xiao, Kai, Yamamoto, & Chen, 2013; Xiao, Muzashvili, & Georgiev, 2014; Xiao, Ni, Kai, & Chen, 2013, in press).

Evidence from epidemiological studies suggests that there is powerful evidence linking dietary polyphenols consumption with the risk factors defining type 2 diabetes, even if some opposite results occurred (Liu et al., 2014; van Dam, Naidoo, & Landberg, 2013). However, how dietary polyphenols impact major endpoints of type 2 diabetes are still inconclusive due to the complex relationships among the risk factors of type 2 diabetes and the high number of dietary polyphenols (including their metabolites) present in the diet.

Almost all polyphenols show anti-oxidant potential which most likely mediate their beneficial health effects associated with cancer, diabetes, hypertension, cardiovascular and neurodegenerative diseases, aging, and so on (Thomas & Pfeiffer, 2012; Xiao, 2013). In this context, dietary polyphenols are proved to be highly active and are considered as viable alternatives to conventional drugs for various free radicals mediated diseases. However, the influence

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of high levels of glucose and glycated plasma proteins in diabetics on the free radical scavenging potential of natural antioxidants in blood is not clear. Herein, taking advantage of several anti-oxidative assays involving 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical, 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) free radical, and superoxide anion radical scavenging and ferric reducing antioxidant potential (FRAP) assay, the difference between healthy human plasma proteins (HPP) and type 2 diabetes plasma proteins (TPP) on the antioxidant potential of dietary polyphenols was compared. Fifty dietary polyphenols (Table 1) were studied.

2. Materials and methods

2.1. Chemicals

Biochanin A, genistein, apigenin, puerarin, catechin (C), epicatechin (EC), luteolin, GCG, DPPH, ABTS, EGCG, EGC, ECG, nitrotetrazolium blue chloride (NBT), potassium persulfate, and phenazine methosulfate (PMS) were purchased from Aladin Co. Ltd. (Shanghai, China). Nicotinamide adenine dinucleotide (NADH) was obtained from Sangon Biotech Co. Ltd (Shanghai, China). Flavone, chrysin, and baicalein (99.5%) were obtained commercially from Wako Pure Chemical Industries (Osaka, Japan). Flavanone, 7-hydroxyflavanone, 6-hydroxyflavanone, 6-methoxyflavanone, 6-hydroxyflavone, 6-methoxyflavone, naringenin, quercetin, and fisetin naringenin were purchased from TCI Chemical Industries (Tokyo, Japan). The other polyphenols were obtained commercially from Shanghai Tauto Biotech CO., Ltd (Shanghai, China). The working solutions of polyphenols (1.0×10^{-3} M) were prepared by dissolving each polyphenol in methanol. All other reagents and solvents were of analytical grade and all aqueous solutions were prepared using freshly double-distilled water.

2.2. Apparatus

UV Absorbance was read on a TU-1810 UV-spectrophotometer (Beijing, China). The fluorescence spectra were recorded on a

Table 1
The chemical structure of natural polyphenols tested in this study.

Subclass	Name	Substitutions		
		OH	OCH_3	Others
Flavones	Flavone			
	7-Hydroxyflavone	7		
	6-Hydroxyflavone	6		
	6-Methoxyflavone		6	
	7,8-Dihydroxyflavone	7,8		
	Baicalein	5,6,7		
	Chrysin	5,7		
	Baicalin	5,6		7-β-D-glucuronide
	Luteolin	5,7,3′, 4′		
	Wogonoside	5	8	7-O-glucuronide
Flavonols	Galangin	3,5,7		
	Kaempferide	3,5,7	4′	
	Kaempferol	3,5,7, 4′		
	Quercetin	3,5,7,3′, 4′		
	Quercitrin	5,7,3′, 4′		3-O-β-D-glucoside
	Myricetin	3,5,7,3′, 4′, 5′		
	Myricetrin	5,7,3′, 4′, 5′		3-O-rhamnoside
	Fisetin	3,7,3′, 4′		
	Rutin	5,7,3′, 4′		3-α-L-Rham-1,6-D-Glc
Isoflavones	Formononetin	7	4′	
	Genistein	5,7,4′		
	Daidzein	7,4′		
	Daidzin	4′		7-Glucoside
	Genistin	5,4′		7-Glucoside
	Biochanin A	5,7	4′	
	Puerarin	7,4′		8-C-glucoside
	Sophoricoside	5,7		4′-O-glucoside
Flavanone	Naringenin	5,7, 4′		
	Naringin	5,4′		7-Neohesperidose
	Hesperidin	5,7, 3′	4′	
	Hesperitin	5,3′	4′	7-α-L-Rham-1,6-D-glc
	Dihydromyricetin	3,5,7,3′, 4′, 5′		
	Flavanone			
	7-Hydroxyflavanone	7		
	6-Hydroxyflavanone	6		
	Silibinin			
	4′-Hydroxyfavanone	4′		
	Neohesperidin	5′	4′	7-O-neohesperidoside
Flavanonol	GCG (2,3-trans)	5,7,3′,4′,5′		3-Gallate
	EGCG (2,3-cis)	5,7,3′,4′,5′		3-Gallate
	ECG (2,3-cis)	5,7,4′,5′		3-Gallate
	EGC (2,3-cis)	3,5,7,3′,4′,5′		
	EC (2,3-cis)	3,5,7,4′,5′		
Stilbenes	Resveratrol	3,5,4′		
	Polydatin	5,4′		3-Glucoside

JASCO FP-6500 fluorometer (Tokyo, Japan). The pH measurements were carried out on a Cole-Parmer PHS-3C Exact Digital pH-meter (Vernon Hills, IL).

2.3. Collection of plasma proteins samples

Serums from type 2 diabetics and health volunteers were obtained from Shanghai Dahua Hospital (Shanghai, China). The routine blood count, blood glucose, glycated albumin and glycated hemoglobin in serums were analysed in Shanghai Dahua Hospital. The levels of glucose and glycated hemoglobin in serums from type 2 diabetics were defined as higher than 6.10 mM and 6.3%, respectively. The levels of glucose and glycated hemoglobin in bloods from healthy adult volunteers were defined as lower than 6.10 mM and 6.3%. The concentrations of total protein, albumin and globulin were within the range of 58.10–70.10 g/L, 27.5–39.4 g/L and 24.0–30.9 g/L, respectively (the detailed data were not provided here). The blood was allowed to clot in glass centrifuge tubes for 2–4 h to obtain serum. Serums from type 2 diabetics and health volunteers were centrifuged at 3000 rpm for 10 min to separate serum from the blood cells to get TPP and HPP, respectively.

2.4. Antioxidant assay

For antioxidant assay, the polyphenols in the absence and presence of serums (TPP or HPP) from three different type 2 diabetics and health volunteers were tested and the average results were reported. The working solutions of TPP and HPP were prepared by directly diluting above serums 1:200 with double distilled water. One hundred microlitres of polyphenols (1.0×10^{-3} M) were mixed with 900 μl of TPP (1:200) or HPP (1:200) and incubated at 37 °C for 30 min to prepare the samples for test. The control group consisted of 100 μl polyphenol and 900 μl of double distilled water.

2.4.1. DPPH free radical scavenging activity

The DPPH free radical scavenging activity of dietary polyphenols in the absence and presence of serums (TPP or HPP) was measured according to literatures with minor modifications (Cao, Chen, & Yamamoto, 2012; Ren, Qiao, & Ding, 2013). One hundred microlitres of samples were added to 900 μl of DPPH solution (1 mM in 50% ethanol). Following incubation in dark for 30 min, the absorbance at 517 nm was measured. The DPPH free radical scavenging potential was calculated using the following formula:

$$\text{DPPH free radical scavenging activity } (\%) = (1 - A1/A0) \times 100 \quad (1)$$

where A_0 is the absorbance of the control, and A_1 is the absorbance of samples. Each sample was tested three times ($n = 3$). The absorbance was found to be reproducible within experimental errors.

2.4.2. ABTS assay

The ABTS assay described by Re et al. was used with minor modifications (Arts et al., 2002; Re et al., 1999). The ABTS assay assesses the total radical scavenging capacity based on the ability of an antioxidant to scavenge the stable ABTS radical cation ($ABTS^{\cdot+}$), which was produced by mixing 1.0 ml ABTS stock solution (7.4 mM) with 1.0 ml potassium persulfate (2.6 mM) and allowing to stand in the dark at room temperature for 12 h before use. The $ABTS^{\cdot+}$ solution was diluted 50 times with PBS (pH 7.4) at 37 °C. Samples (100 μl) were added to 900 μl of $ABTS^{\cdot+}$ diluted solution. Following kept in the dark at room temperature for 60 min, the absorbance was spectrophotometrically determined at 734 nm. The $ABTS^{\cdot+}$ scavenging activity was calculated using the following formula:

$$\text{ABTS radical scavenging activity } (\%) = (1 - A1/A0) \times 100 \quad (2)$$

where A_0 is the absorbance of the control, and A_1 is the absorbance of sample. Each sample was tested three times ($n = 3$). The absorbance was found to be reproducible within experimental errors.

2.4.3. Superoxide anion radical (O^{2-}) scavenging activity

Superoxide anion (O^{2-}) scavenging activity of polyphenols in the absence and presence of serums was measured by Chun et al. with slightly modification (Chun, Kim, & Lee, 2003; Nishikimi, Appaji Rao, & Yagi, 1972). The superoxide radical was generated in 1.0 ml of sodium phosphate buffer (100 mM, pH 7.4) containing 300 μl NBT (50 μM) solution, 300 μl NADH (78 μM) solution and samples (10 μM). The reaction started by addition of 300 μl PMS (10 μM). Following incubation at room temperature for 8 min, the absorbance at 560 nm was measured. The capability to scavenge the superoxide radical was calculated using the following equation:

$$O^{2-} \text{ scavenging activity } (\%) = (1 - A1/A0) \times 100 \quad (3)$$

where A_0 is the absorbance of the control only without sample, and A_1 is the absorbance of sample. Each sample was tested three times ($n = 3$). The absorbance was found to be reproducible within experimental errors.

2.4.4. Ferric reducing power (FRAP) assay

The FRAP assay was performed as described previously (Li, Feng, Huang, & An, 2013). The FRAP reagent was freshly prepared each day by mixing 300 μl acetate buffer (pH 3.6, 0.30 M), 300 μl TPTZ (10 mM) and 300 μl iron(III) chloride (20 mM). Sample (100 μl) was added to above FRAP reagents. Following incubation in dark for 30 min, the absorbance at 593 nm was measured. Each sample was tested three times ($n = 3$). The absorbance was found to be reproducible within experimental errors.

2.5. Fluorescence spectra

The binding affinities of polyphenols for plasma proteins were determined by fluorescence spectra as described by Wang et al. (2012). The working solutions of TPP and HPP were prepared by directly diluting above serums with double distilled water (1:100). Three millilitres plasma proteins working solution was transferred to a 1.0 cm quartz cell. Then it was titrated with successive addition of 3.0 μl polyphenol solution (1.0×10^{-3} M). In each titration, the fluorescence spectrum was collected with the working solution of plasma proteins. The fluorescence spectra of plasma proteins working solution were measured in the wavelength range of 310–450 nm upon excitation at 280 nm and the fluorescence intensities of at 336.25 nm were recorded.

The binding constants were calculated according to the double-logarithm equation (34–37):

$$\lg((F_0 - F)/F) = \lg K_a + n \lg(Q) \quad (4)$$

where F_0 and F represent the fluorescence intensities of TPP or HPP work solution at 336.25 nm under excitation at 280 nm in the absence and in the presence of polyphenols, K_a is the binding constant, n is the number of binding sites, and (Q) is the concentration of polyphenols.

3. Results

3.1. DPPH free radical scavenging potential of polyphenols in the presence of plasma proteins

DPPH assay is based on the measurement of the scavenging ability of antioxidants towards the stable DPPH free radical. It is considered a valid and easy assay to evaluate radical-scavenging activity of antioxidants. Fig. 1 showed the DPPH free radical scavenging potential of tested polyphenols in the presence and absence of plasma proteins. Polyphenols such as 4′-hydroxylfavanone, wogonoside, 7-hydroxylflavone, flavanone, 7-hydroxylflavanone, 6-methoxylflavanone, flavone, 6-hydroxylflavanone, and 6-hydroxylflavone hardly scavenged the DPPH free radical. Moreover, silibinin, puerarin, biochanin A, sophoricoside, genistein, daidzein, daidzin, genistin, galangin, hesperidin, hesperitin, and polydatin appear very low scavenging potential against the DPPH free radical. These polyphenols with relative low DPPH free radical scavenging percentages (<50%) were sorted as group Φ1, and others were classified as group Φ2 (Fig. 1).

As shown in Fig. 1, both HPP and TPP apparently masked the dietary polyphenols in group Φ2, thus reducing the DPPH radical scavenging potential. Plasma proteins hardly masked polyphenols in group Φ1. Compared with HPP, TPP significantly weakened the masking effect on the polyphenols in group Φ2, and thus improved their DPPH free radical scavenging capacity of (Fig. 1). For example, HPP decreased about 24.2%, 24.1%, and 20.9% DPPH free radical scavenging potential of myricetin, quercetin, and luteolin, respectively; however, TPP only reduced about 6.7%, 9.3%, and 6.6% DPPH free radical scavenging potential of myricetin, quercetin, and luteolin.

3.2. ABTS radical scavenging of polyphenols in the presence of plasma proteins

ABST assay is based on the measurement of the ability of antioxidants towards scavenging the stable ABTS radical. Polyphenols appear to fast react with ABTS radical. The ABTS radical scavenging activities of polyphenols (final concentration 1.0 μM) in the absence and presence of plasma proteins are shown in Fig. 2. Among polyphenols tested, only GCG, EGC, EGCG, gallic acid, quercetin, pyrogallic acid, ECG, myricetin, fisetin, luteolin, 7,8-dihydroxyflavone, quercetrin, methyl gallate, myricetrin, ethyl gallate, bacalein, dihydromyricetin, and propyl gallate showed relative strong ABTS radical scavenging potential (Fig. 2, Group Φ1). Other polyphenols (Group Φ2) showed relative weak ABTS radical scavenging potential. As shown in Fig. 2, it illustrated that both HPP and TPP apparently masked the dietary polyphenols in Group Φ1, thus reducing their ABTS radical scavenging capacities. For example, HPP reduced about 13.0%, 27.0%, and 16.6% of ABTS radical scavenging potential of GCG, EGC, and EGCG, respectively; however, TPP also weakened about 9.3%, 23.9%, and 13.5% ABTS radical scavenging potential of GCG, EGC, and EGCG.

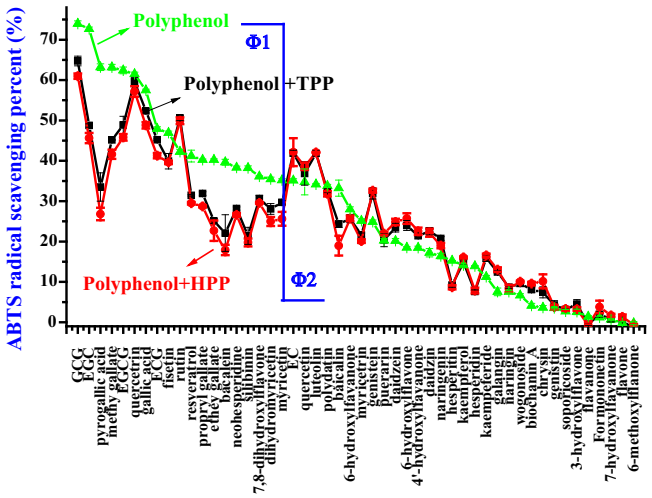


Fig. 2. Plasma proteins impact ABTS radical scavenging potential of polyphenols.

3.3. Superoxide anion radical scavenging of polyphenols in the presence of plasma proteins

Fig. 3 showed the inhibitory activity of polyphenols in the absence and presence of plasma proteins on superoxide anion radical generation. Among these polyphenols (final concentration 1.0 μM) tested, only GCG, EGCG, myricetin, EGC, ECG, dihydromyricetin, myricetrin, rutin, 7,8-dihydroxyflavone, gallic acid, pyrogallic acid, quercetrin, ethyl gallate, propyl gallate, fisetin,

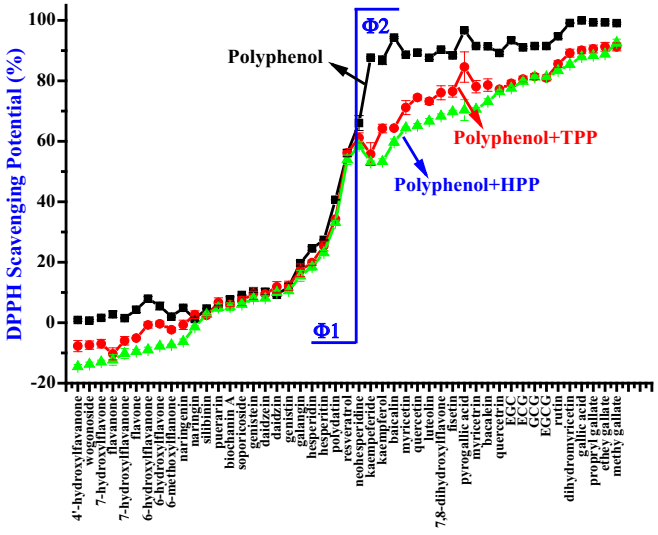


Fig. 1. Plasma proteins influence DPPH free radical scavenging potential of polyphenols.

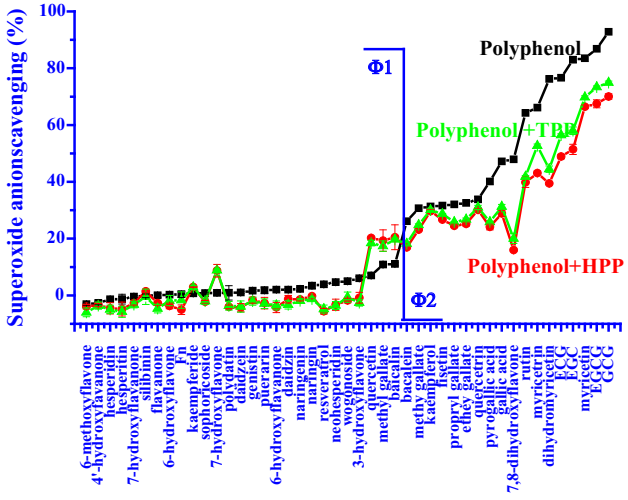


Fig. 3. Plasma proteins affect superoxide anion radical scavenging potential of polyphenols.

kaempferol, methyl gallate, and bacailein showed relative strong O^{2-} radical scavenging potential (>20%) (Fig. 3, Group Φ2). Others (Group Φ1) hardly scavenged O^{2-} radical. As shown in Fig. 3, both HPP and TPP apparently masked dietary polyphenols in Group Φ2, thus reducing O^{2-} radical scavenging potential. However, both HPP and TPP hardly influenced O^{2-} radical scavenging potential of dietary polyphenols in Group Φ1. Furthermore, it illustrated that compared with HPP, TPP reduced the masking effect on polyphenols in Group Φ2. For example, HPP significantly reduced about 27.7%, 31.6%, 19.3%, and 22.8% of O^{2-} radical scavenging activities of ECG, EGC, EGCG, and GCG, respectively; however, TPP only decreased about 20.2%, 25.0%, 13.4%, and 17.9% O^{2-} radical scavenging activities of ECG, EGC, EGCG, and GCG.

3.4. Ferric reducing power of polyphenols in the presence of plasma proteins

The clinical utility of measuring antioxidant or reducing power in biological fluids remains to be established. A simple, automated test measuring the ferric reducing ability of polyphenols, the FRAP assay, is presented as a method for assessing "antioxidant power" (Çekiç, Çetinkaya, Avan, & Apak, 2013). Ferric to ferrous ion reduction at low pH leads to a coloured ferrous-tripyridyltriazine complex to form. FRAP values are obtained by comparing the absorbance change at 593 nm in test reaction mixtures with those containing ferrous ions in known concentration. Fig. 4 showed the reductive influence of plasma proteins on the ferric reducing power of polyphenols (expressed as concentration of Fe^{2+} (mM)). It suggested that serums in most case hardly influenced the ferric reducing power of polyphenols.

3.5. Affinity–activity relationship of plasma protein–polyphenols systems as free radical scavengers

The binding constants were calculated by using the logarithmic form of the Stern–Volmer equation. According to Eq. (4), the values of "$(F_0 - F)/F$" can be obtained in each "(Q)". And then, the linear regression equation between the "$\lg((F_0 - F)/F)$" values and "$\lg(Q)$" values was obtained on the Origin 7.5 software (Wang et al., 2012). The slope factor means "n" and the intercept refers to "$\lg K_a$". Each polyphenol was tested with three HPP or TPP samples from different people and the average binding constant and

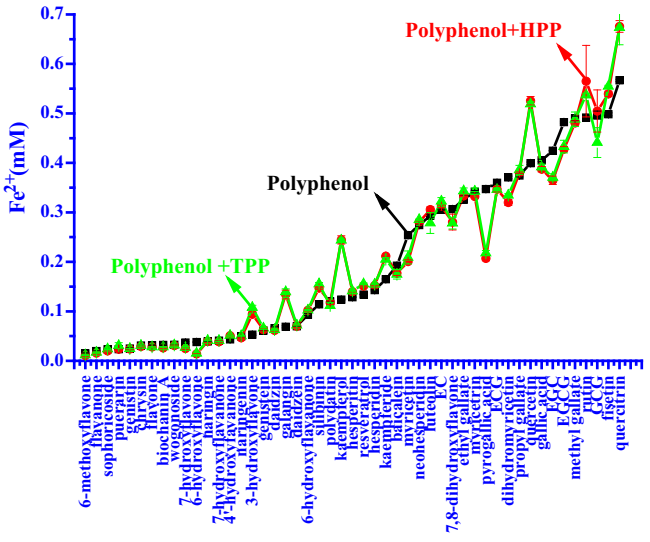


Fig. 4. Impact of plasma proteins on FRAP of polyphenols.

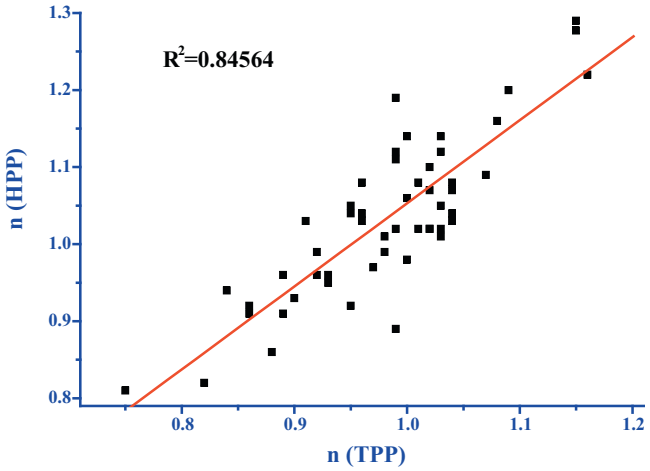


Fig. 5. The relationship between binding affinities of polyphenols for HPP and TPP.

the number of binding sites are obtained (Fig. 5). The affinities of polyphenols especially for flavonoids to plasma proteins are mainly correlated with structural motifs of the molecules, frequently invoked as affecting their reactivity and activity in a variety of physiological processes. Scheme 1 showed the role of structure elements on the affinity for plasma proteins via their influence on some molecular properties. These structural motifs are as follows (Xiao & Kai, 2012; Cao, Shi, & Chen, 2013; Xiao et al., 2011):

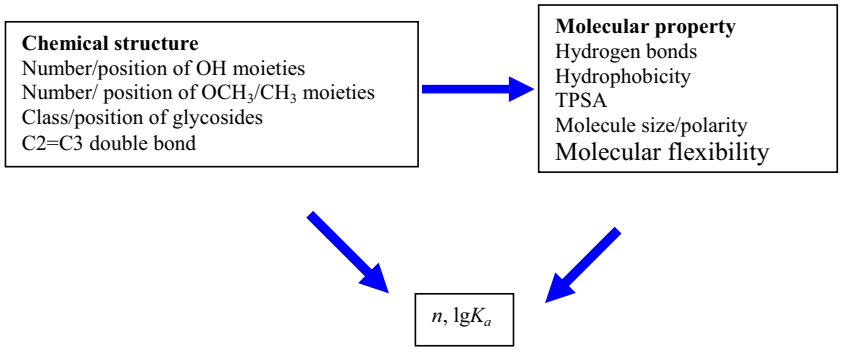
(a) The number and position of the hydroxyl groups in the rings A, B, and C.
(b) The methoxyl or methyl groups in the rings A and B.
(c) The class and position of the glycosides.
(d) The degree of saturation of the ring C (the presence or absence of a C2–C3 double bond).

Fig. 5 showed the relationship between binding affinities (the number of binding sites) of polyphenols for HPP and TPP. The values of n (polyphenol–HPP) are directly proportional to the values of n (polyphenol–TPP) with linear relationship (R^2 = 0.84564). The affinities of flavonoids for HPP and TPP were within the range of 10^4–10^6 L/mol, which were obviously much lower than the affinities for purified HSA from our previous reports (10^4–10^8 L/mol) (Xiao & Kai, 2012; Cao et al., 2013; Xiao et al., 2011).

We further investigated the affinity–activity relationship of plasma protein–polyphenols complex as free radical scavengers. Fig. 6 showed the relationship between the binding affinities of polyphenols for plasma proteins and the masked DPPH free radical scavenging capacities of polyphenols by HPP and TPP. There are no obvious affinity–activity relationships of plasma protein–polyphenols plasma protein–polyphenols systems as DPPH free radical scavengers (R^2 = 0.09635 for HPP and R^2 = 0.11333 for TPP). Moreover, there is no relation between the binding affinities of polyphenols for plasma proteins and the masked ABTS scavenging capacities of polyphenols (Data were not shown here).

4. Discussion

The difference between HPP and TPP on the binding affinity with dietary polyphenols was investigated in detailed by our group by fluorescence spectroscopy (Xie et al., 2012). The non-covalent interaction between small molecules and proteins usually caused by four major interaction forces, namely, hydrogen bonding force, van der Waals force, hydrophobic interaction and electrostatic interaction.



Scheme 1. Structural and molecular properties of polyphenols that influences the affinity for plasma proteins.

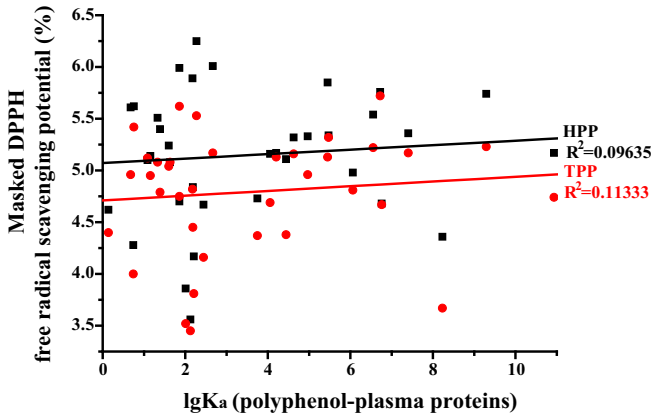


Fig. 6. The relationship between the binding affinities of polyphenols for plasma proteins and the masked DPPH scavenging capacities of polyphenols by HPP and TPP.

The nature of polyphenol–TPP and polyphenol–HPP interaction was studied by investigation the molecular property–affinity relationship. It suggested that TPP showed about 1–10 times lower affinities with polyphenols than HPP. The values of lgK_a(HPP) are positive proportional to the values of lgK_a(TPP) with excellent linear relationship (Xiao & Kai, 2012; Cao et al., 2013; Xiao et al., 2011). The glycation of HPP decreased the affinities for HPP about 1.17–16.6 times (Cao et al., 2013; Xiao et al., 2011; Xie et al., 2012). The difference between HPP–polyphenol interaction and TPP–polyphenol interaction was bigger for the more lipophilic polyphenols. The affinities for TPP or HPP SLIGHTLY decreased with increasing hydrogen bond donor numbers of polyphenols and HARDLY changed with hydrogen bond acceptor numbers (Cao et al., 2013; Xiao et al., 2011; Xie et al., 2012).

Normal cellular activities involving many physiological processes may yield reactive oxygen species (ROS), such as O^{2-} and OH·. The purpose of antioxidants such as polyphenols is to prevent ROS from reaching to a high level within the cell. Antioxidants are considered as a favourable therapy for these diseases related to ROS (Wang & Goodman, 1999).

Several assays are available to evaluate the antioxidant potential of polyphenol–protein systems *in vitro*. However, in most of the reports, authors have mentioned that use of one assay for an individual study. For example, the radical scavenging/antioxidant potentials measured by ABTS and FRAP assays do not correlate well because differences in reactive chemistry of the analyte may favour one or the other assay (Chandra & Uchimaru, 2002). Hagerman et al. recently reported there is little linear relationship between the response of the various polyphenols in the ABTS assay and FRAP assay (Zou & Xie, 2013).

In current study, there is also consistency to a certain degree between the affinity and the free radical scavenging capacities of polyphenols–plasma proteins system (Fig. 6). TPP showed lower binding affinities for polyphenols in blood than that of HPP, which results in releasing more free polyphenols. It illustrated that serum proteins masked the dietary polyphenols, thus reducing their free radical scavenging potential, such as DPPH free radical, ABTS radical, and O^{2-} radical scavenging. Compared with HPP, TPP obviously increased the antioxidant capacity of polyphenols.

The corresponding consequence of increased glycation of plasma proteins in diabetics is to improve free polyphenols in blood, which causes polyphenols expose to free radicals in blood. Therefore, polyphenols in diabetics' blood are tend to be oxidised and cannot be efficiently delivered to other tissues, which reduces the beneficial impact of polyphenols. It suggested that the oxidant-scavenging ability of blood is most probably a result of the synergistic effects exerted by the free circulating antioxidants, antioxidant–protein complexes, blood cells coated with antioxidants, and so on. An understanding of diabetes-associated changes in absorption, disposition, metabolism, elimination and bioactivities of dietary polyphenols as well as the mechanism of the variability should lead to the improvement of the benefits of these polyphenols and clinical outcomes for diabetics. It is interesting to improve the beneficial effects of dietary polyphenols and clinical outcomes for diabetics and to understand how to use dietary polyphenols for diabetics. A good understand of diabetes-associated pharmacological changes for dietary polyphenols will helpful to develop methods to improve their efficiency for preventing and managing type 2 diabetes. The further research on assessing why glycated plasma proteins are able to cause a big difference on oxidant-scavenging potential of polyphenols, which could help in changing the way such polyphenols are used in the treatment of diabetes. Furthermore, we should pay more attention to *in vivo* benefits of dietary polyphenols for preventing and managing type 2 diabetes. Recently, we investigate the structure–activity relationship of dietary polyphenols inhibiting the glycation of bovine serum albumin to explore related mechanisms. It illustrated that the polyphenols with higher affinity with BSA showed stronger inhibition against the glycation of BSA. Polyphenols appear strong the free radical scavenging potential, which can protect BSA from glycation.

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