Journal of Cell Science & Therapy

Postal, et al., J Cell Sci Ther 2014, 5:3
DOI: 10.4172/2157-7013.1000162

Research Article Open Access

Mechanism of Action of Nutraceuticals on Intestine to Ameliorate Glucose Homeostasis: Follow-up Studies by an *In Situ* Approach

Bárbara Graziela Postal[1], Samantha Manes Guesser[1], Virgínia Demarchi Kappel[1], Ana Paula Ruani[2], Nicolas Suarez Zamorano[1,3], Angela Machado de Campos[4], Flávio Henrique Reginatto[4], Moacir Geraldo Pizzolatti[2], Daniela Ota Hisayasu Suzuki[5] and Fátima Regina Mena Barreto Silva[1*]

[1]Department of Biochemistry, Biological Sciences Center, Federal University of Santa Catarina, Trindade Neighborhood, Cx Postal 5069, CEP: 88040-970 - Florianópolis, SC, Brazil

[2]Departamento of Chemistry, Center of Physical and Mathematical Sciences, Federal University of Santa Catarina, University Campus, Bairro Trinity Cx. Postal 5069, ZIP: 88,040 to 970 - Florianópolis, SC, Brazil

[3]Pierre and Marie Curie University, Sorbonne University, Paris 6, Place Jussieu - 75252 Paris Cedex 05, France

[4]Department of Pharmaceutical Sciences, Health Sciences Center, Federal University of Santa Catarina, Florianópolis, SC, Brazil

[5]Department of Electrical Engineering, Institute of Biomedical Engineering, Federal University of Santa Catarina, University Campus, Trindade neighborhood, Florianópolis, SC, Brazil

[*]**Corresponding author:** Fátima Regina Mena Barreto Silva, Department of Biochemistry, Biological Sciences Center, Federal University of Santa Catarina, Florianópolis, SC, Brazil, Tel: +55-48.3721.69.12; Fax: +55-48.3721.96.72; E-mail: mena.barreto@ufsc.br

Rec date: Mar 28, 2014; **Acc date:** Apr 11, 2014; **Pub date:** Apr 14, 2014

Abstract

Background: Epidemiologic studies and clinical trials have suggested a correlation between dietary polyphenols and prevention of chronic diseases such as diabetes. The current study has been limited to the compounds previously studied by *in vivo* and *in vitro* experimental model concerned glucose homeostasis.

Objective: Some selected compounds as myricitrin, quercetin, catechin, naringenin, caffeic acid, rutin, fukugetin, hispidulin, kaempferitrin and chlorogenic acid were investigated in sodium-glucose co-transporter activity in rat intestine through an *in situ* approach, compared with phlorizin, a classical competitive inhibitor of Na^+-dependent glucose transport. Methods: For the *in situ* studies the intestine segments were uploaded with glucose solution, phlorizin and/or compounds and after 30 min the glucose was measured into the respective intestinal segment.

Results: Among the substances assayed, myricitrin, quercetin, catechin, naringenin, caffeic acid, rutin and fukugetin significantly reduced the glucose uptake by affecting the SGLT1 transporter activity measured in the presence of phlorizin. It worthwhile mentions that myricitrin at 10mM exhibited a *per se* inhibitory effect around 90% higher than that observed for phlorizin. Quercetin inhibited the glucose uptake in both concentrations used and exhibited an effect on glucose absorption as good as phlorizin at 10 mM. Catechin and caffeic acid (10 mM) in the presence of phlorizin potentiated the inhibitory effect of this compound on glucose uptake. Moreover, 10 mM naringenin showed a similar inhibitory effect of phlorizin. Additionally, rutin and fukugetin (10 mM) alone or in combination with phlorizin slightly reduced the glucose absorption.

Conclusion: Based on these results, myricitrin, quercetin, catechin, naringenin, caffeic acid, rutin and fukugetin are able to regulate glucose absorption by acting in an intestinal target, SGLT1, contributing to ameliorate glucose homeostasis.

Keywords: SGLT1; Glucose absorption; Flavonoids; Caffeic acid; Polyphenois; Glycemia

Introduction

The dietary glucose cannot cross easily the lipid intestinal bilayer of enterocyte. So, specific transporters are required for glucose intestinal absorption. The Na^+/glucose co-transporter (SGLT1) at the enterocyte apical membrane transports glucose into the cell against its concentration gradient. Also, a facilitative Glucose Transporter (GLUT2), transports sugars across the basolateral membrane to the blood [1,2]. Some studies show evidences that GLUT2 can also be recruited to the Brush-Border Membrane (BBM) in the presence of high glucose concentration in the lumen and can participate in the intestine glucose absorption process [3].

SGLT1 has high affinity for glucose and transport two sodium ions for each molecule of glucose. It was postulated that the active glucose transport through the intestinal epithelium is driven by the sodium gradient across the membrane, by means of a Na^+/glucose co-transport [4]. It is believed that Na^+ and glucose transport by the SGLT1 occur by a sequential mechanism in which two Na^+ ions from the extracellular side bind to the transporter just before the glucose induces a conformational change in the glucose binding site and increases the transporter affinity for the substrate. Also, together with the Na^+ and glucose transport the SGLT1 promotes depolarization at plasma membrane, which can serve as a signal. In addition, in the absence of glucose the SGLT1 can support a Na^+ current [4,5]. Many studies suggest that polyphenols such as phlorizin and phloretin decrease intestinal glucose absorption [6]. Phlorizin, a flavonoid glycoside found in apples, is known as a competitive inhibitor of Na^+-

dependent glucose transport across of BBM [7]. Also, phloretin is the aglycone of phlorizin and an inhibitor of intestinal glucose transport mediated by GLUT2 [8].

Polyphenols are secondary metabolites of plants and found largely in fruits, vegetables, cereals and beverages, thus form an integral part of the human diet. Several biological activities and beneficial properties are described for dietary polyphenols and it has suggested that long term consumption of diets rich in plant-foods polyphenols, including phenolic acids and flavonoids, is associated with some protection against a variety of disease states [9,10]. In recent years, the interest in polyphenols as nutraceuticals and supplementary treatments for various aspects of diabetes mellitus has widely increased [11]. It was showed that dietary plant polyphenols and polyphenol-rich products modulate carbohydrate and lipid metabolism, attenuate hyperglycemia, dyslipidemia and insulin resistance, improve adipose tissue metabolism, and can also prevent the development of long-term diabetes complications [12]. However, the mechanism of action of nutraceuticals in the intestine is not completed understood. So, taking it in account and our previous studies concerned with glycemia control by several natural compounds [9,13-16] also deeply discussed in two chapters [2,11], the aim of the present study was to investigate the acute effect of some selected natural compounds (Figure 1) on glucose transporter, SGLT1, by *in situ* treatment.

Materials and Methods

Chemicals

Phlorizin, caffeic acid, chlorogenic acid and glucose were purchased from Sigma Chemical Company® (St. Louis, MO, USA). Ketamine was purched from AgenerUnião (Embu-Guaçu, SP, Brazil) and xylazine was purchased from Bayer (Leverkusen, Germany).

Plant material

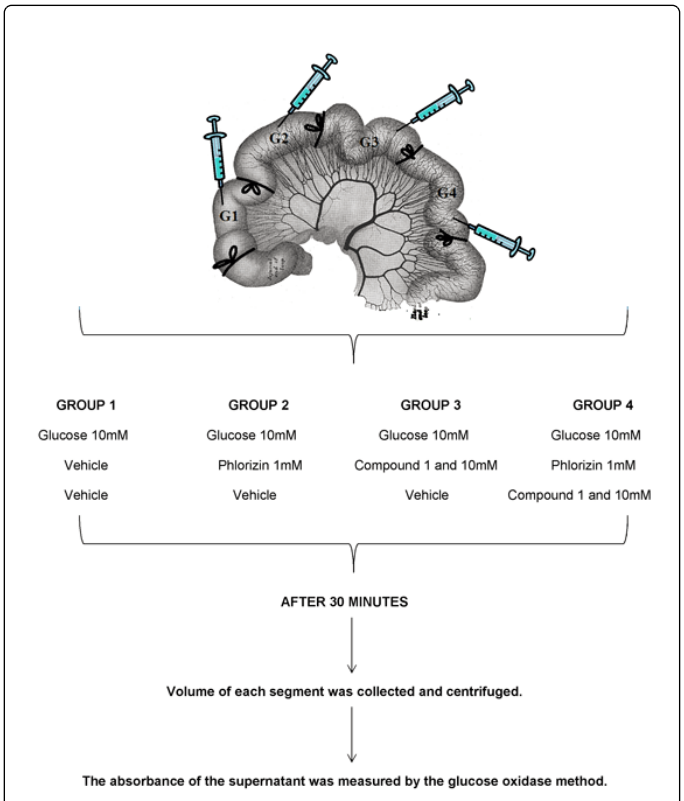
Kaempferitrin was isolated from leave of the *Bauhinia forficata* Link [17], quercetin, hispidulin and naringenin were isolated from *Baccharis pseudotenuifolia* [18], catechin was obtained from bark of the *Croton celtidifolius Baill* [19], fukugetin was isolated from leaves of the *Rheedia gardneriana* [20], Rutin was obtained from *Polygala paniculata* [21] and myricitrin was isolated from leaves of the *Eugenia uniflora* [22].

Animals

Male Wistar rats (180–200g) were bred in animal facility and housed in an air-conditioned room (approximately 20 ± 2°C) with a controlled 12: 12 h light/dark cycle (lights on from 06: 00 to 18: 00 h). The animals were maintained with pelleted food (Nuvital, Nuvilab CR1, Curitiba, PR, Brazil) and tap water was available ad libitum. Fasted animals were deprived of food for at least 16 h but allowed free access to water. All the animals were monitored and maintained in accordance with the ethical recommendations approved by the Committee for Ethics in Animal Research of UFSC (Protocol CEUA n ° PP00398). For glucose absorption, briefly, rats received anesthesia (ketamine/xylazine; 75/10 mg/kg) association by intraperitoneal injection according Gonzalez-Mujica et al. [23].

Glucose intestinal absorption studies

For each *in situ* experiment, the intestine was exposed and divided in four segments of 4 cm each one by ligatures (Scheme 1). So, 1mL of glucose solution 10 mM was administered by a syringe in each divided portion (segment 1, 2, 3 and 4). Also, in some segments, glucose solution in the presence (segment 2 and 4) and absence of 1 mM of phlorizin (segment 1 and 3) were used. For treated groups, a glucose solution plus compounds in the absence (segment 3) and presence (segment 4) of phlorizin were assayed. Following 30 min the content of each segment was collected and glucose was measured by glucose-oxidase method [24]. Any significant change in volume collected was observed compared with the initial volume added. The assays were carried out according Gonzalez-Mujica et al. [25].



Scheme 1: Absorption of glucose was measured from four exposed parts of intestine by glucose oxidase method

In situ basal glucose absorption (10 mM) was measured at 5, 10, 15 and 30 min. Glucose absorption in the presence of phlorizin (1 mM) at 30 min was also measured. After that, phlorizin, a SGLT1 inhibitor, was assayed with/without different natural compounds at 30 min. The effect of compounds myricitrin, quercetin, catechin, naringenin, caffeic acid, rutin, fukugetin, hispidulin, kaempferitrin and chlorogenic acid (1 and/or 10 mM) on glucose absorption in the presence or absence of phlorizin was studied.

Data and statistical analysis

Data were expressed as means ± S.E.M. One-way analysis of variance (ANOVA) followed by Bonferroni post-test or unpaired Student's t-test were used to determine the significant difference

between the groups. Differences were considered to be significant at $p \leq 0.05$.

Results

Time-course of basal glucose absorption

As it can be seen in Figure 2A, the basal glucose absorption (10 mM) was significantly increased in time-dependent manner from 5 to 30 min. Phlorizin (1 mM) at 30 min was able to block significantly the glucose absorption (Figure 2B). Also, some experiments with 10, 20, 30, 40 and 50 mM of glucose in the presence or absence of catechin and naringenin (10 mM) with/without phlorizin (1 mM) were carried out in order to evaluate the profile of glucose absorption (data not shown). From those data, 10 mM of glucose concentration it was chosen for the next assays since in this condition phlorizin blocked about 100% of glucose absorption.

Inhibitory effect of nutraceutical compounds on SGLT1

Surprisingly, 10 mM myricitrin inhibited glucose absorption around 95%. In addition, this compound exhibited 90% of inhibitory effect of phlorizin on glucose absorption (Figures 3A and 3B). On the other hand, 1 mM myricitrin did not alter the glucose absorption compared with control group.

Figures 4A and 4B shows the inhibitory effect of quercetin in both concentrations used. As it is observed, this flavonoid (10 mM) was as good as phlorizin on glucose absorption inhibition.

In addition, 10 mM catechin, an aglycone flavonoid widely present on the diet, inhibited efficiently the glucose absorption by acting on SGLT1 Na^+-glucose co-transporter on intestine. Beyond, the catechin presented a *per se* inhibitory effect on glucose absorption and also potentiated the well-known phlorizin effect on intestinal SGLT1. However, any alteration on glucose absorption was observed at 1 mM catechin (Figures 5A and 5B).

Figure 6 shows the *in situ* effect of naringenin on intestinal Na^+-glucose cotransporter. As it was expected, phlorizin significantly reduced the glucose absorption about 85% after 30 min of *in situ* exposition. Also, the administration of 1 mM naringenin not changed the glucose absorption (Figure 6A). However, 10 mM naringenin was able to inhibit glucose absorption around 74% (Figure 6B). It worth mention that naringenin prevented the complete inhibitory effect of phlorizin on glucose absorption.

Figures 7A and 7B show the *in situ* effect of caffeic acid on glucose absorption after an acute exposition. Surprisingly, this compound showed an inhibitory effect on glucose transporter in both concentrations tested as much efficient as that exhibited by phlorizin at the same experimental condition. In addition, the 10 mM caffeic acid potentiated the inhibitory effect of phlorizin bypassing the maximum effect of phlorizin on inhibition of glucose absorption measured in its experimental approach. Furthermore, rutin and fukugetin (10 mM) alone or in combination with phlorizin reduced slightly the glucose absorption and also both flavonoids reduced phlorizin inhibitory effect (Figures 8A, 8B and 9).

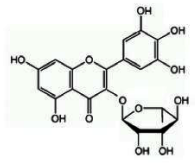
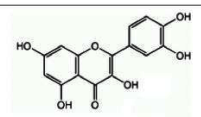
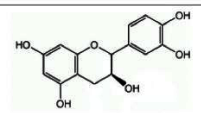
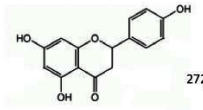
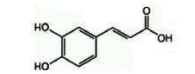
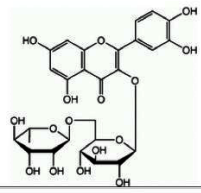
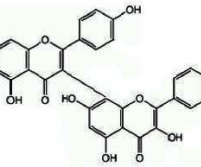
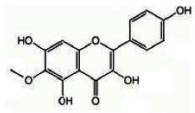
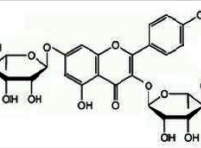
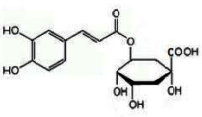
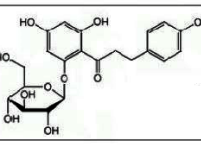
Name	Chemical name (IUPAC)	Chemical structure	M.W.
Myricitrin	4*H*-1-Benzopyran-4-one, 3-[(6-deoxy-α-L-mannopyranosyl)oxy]-5,7-dihydroxy-2-(3,4,5-trihydroxyphenyl)		464.38
Quercetin	4*H*-1-Benzopyran-4-one,2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy		302.24
Catechin	2*H*-1-Benzopyran-3,5,7-triol, 2-(3,4-dihydroxyphenyl)-3,4-dihydro		290.27
Naringenin	4*H*-1-Benzopyran-4-one, 2,3-dihydro-5,7-dihydroxy-2-(4-hydroxyphenyl)	 272,26	272.26
Caffeic Acid	2-Propenoic acid, 3-(3,4-dihydroxyphenyl)		180.16
Rutin	4*H*-1-Benzopyran-4-one, 3-[[6-*O*-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl]oxy]-2-(3,4-dihydroxyphenyl)-5,7-dihydroxy		610.53
Fukugetin	[3,8'-Bi-4*H*-1-benzopyran]-4,4'-dione, 2'-(3,4-dihydroxyphenyl)-2,3-dihydro-5,5',7,7'-tetrahydroxy-2-(4-hydroxyphenyl)-, (2*R*,3*S*)-		568.10
Hispidulin	4*H*-1-Benzopyran-4-one, 5,7-dihydroxy-2-(4-hydroxyphenyl)-6-methoxy-		316.27
Kaempferitrin	4*H*-1-Benzopyran-4-one, 3,7-bis[(6-deoxy-α-L-mannopyranosyl)oxy]-5-hydroxy-2-(4-hydroxyphenyl		578.18
Chlorogenic Acid	Cyclohexanecarboxylic acid, 3-[[3-(3,4-dihydroxyphenyl)-1-oxo-2-propen-1-yl]oxy]-1,4,5-trihydroxy		354.31
Phlorizin	1-Propanone,1-[2-(β-D-glucopyranosyloxy)-4,6-dihydroxyphenyl]-3-(4-hydroxyphenyl)		436.42

Figure 1: Chemical structures of the compounds used in this study

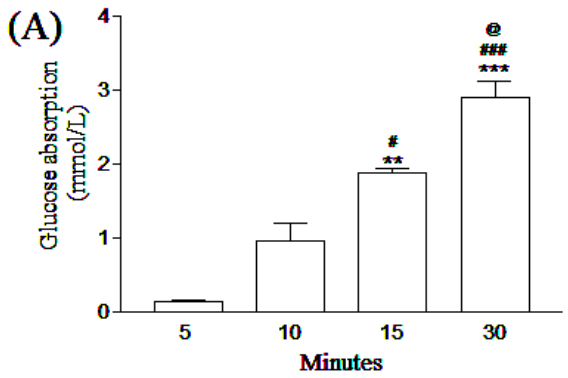


Figure 2A: Time-course of basal glucose absorption. ***p<0.001, **p<0.01, compared with 5 min; ###p<0.001, #p<0.05 compared with 10 min; @p<0.05 compared with 15min

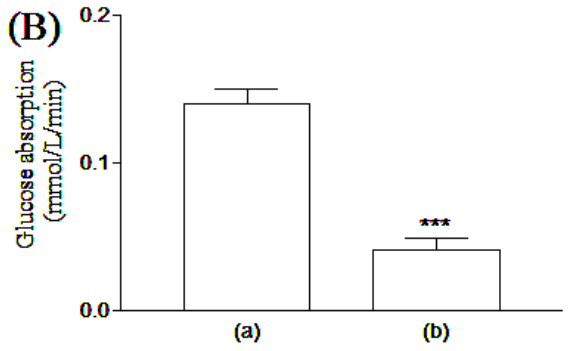


Figure 2B: Effect of phlorizin on glucose absorption at 30 min. 10 mM Glucose (a); 10 mM glucose plus: phlorizin 1 mM (b). Values are means ± S.E.M.; n=4 animals in each group. ***p<0.001 compared with control group (a)

Hispidulin, kaempferitrin, and chlorogenic acid already demonstrated to reduce glycemia in different experimental condition [15,25], were assayed *in situ*. However, all of them did not modify the glucose absorption at any dose evaluated (data not shown).

Discussion

The functional integrity of intestine is an important factor to the efficient absorption of glucose and its distribution to other tissues via circulation [26]. As glucose is of hydrophilic nature it is not able to readily diffuse through the plasma membrane. So, two main kinds of protein transporters participate to this sugar transport: facilitative transporters that are variable and approximately proportional to water absorption (glucose carriers) and active transporter proteins that are constant and not associated with water absorption (glucose carriers – SGLTs) [2,27].

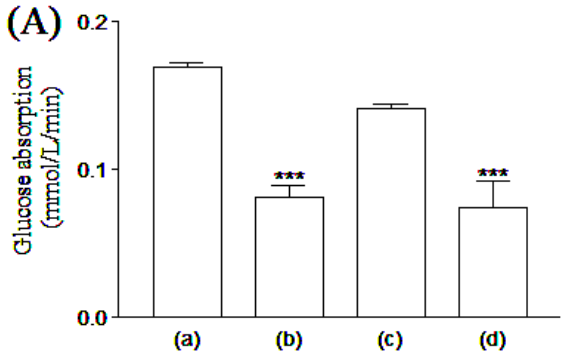


Figure 3A: Effect of myricitrin on intestinal glucose absorption in rat. Measured after 30 min; 10 mM Glucose (a); 10 mM glucose plus 1 mM phlorizin (b), 1 mM myricitrin (c), 1 mM phlorizin plus 1 mM myricitrin (d). Values are means ± S.E.M.; n=4 animals in each group. ***p< 0.001 compared with control group (a)

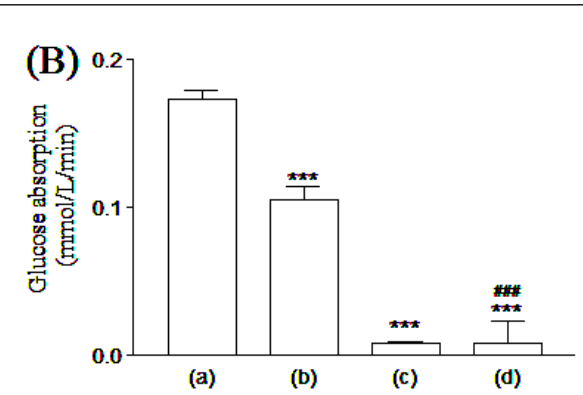


Figure 3B: Effect of myricitrin on intestinal glucose absorption in rat. Measured after 30 min; 10 mM Glucose (a); 10 mM glucose plus 1 mM phlorizin (b), 10 mM myricitrin (c), 1 mM phlorizin plus 10 mM myricitrin (d). Values are means ± S.E.M.; n=4 animals in each group. ***p<0.001 compared with control group (a), ###p<0.001 in relation to phlorizin group

In addition, phlorizin is well known to inhibit Na+-glucose cotransporter and it has been shown that glucose absorption in vivo comprise phlorizin-sensitive and phlorizin-insensitive components [28,29].

In a whole, control over the intestinal and renal reabsorption of glucose constitutes conceivable physiological strategy to achieve the glucose homeostasis and particularly in intestine, SGLT1 is considered a potential target for the drug therapy to glycemic control in diabetic patients [2,5]. The present work was focused on SGLT1 transporter in an *in situ* approach in order to better understand the role of different compounds on glucose absorption.

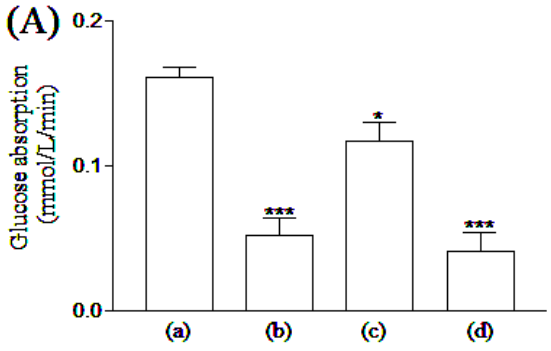


Figure 4A: Effect of quercetin on intestinal glucose absorption in rat. Measured after 30 min; 10 mM Glucose (a), 10 mM glucose plus 1 mM phlorizin (b), 1 mM quercetin (c), 1 mM phlorizin plus 1 mM quercetin (d). Values are means ± S.E.M.; n=4 animals in each group. *p< 0.05 and ***p< 0.001 compared with control group (a)

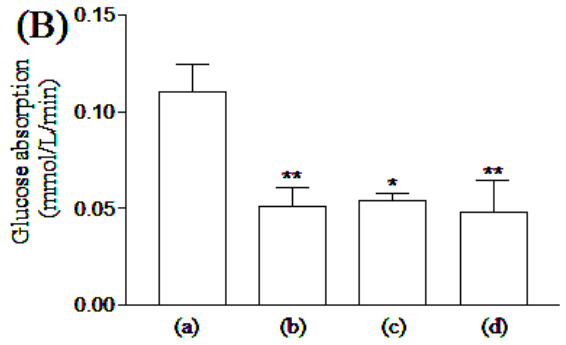


Figure 4B: Effect of quercetin on intestinal glucose absorption in rat. Measured after 30 min; 10 mM Glucose (a); 10 mM glucose plus 1 mM phlorizin (b), 10 mM quercetin (c), 1 mM phlorizin plus 10 mM quercetin (d). Values are means ± S.E.M.; n=4 animals in each group. *p<0.05, **p<0.01 and ***p<0.001 compared with control group (a)

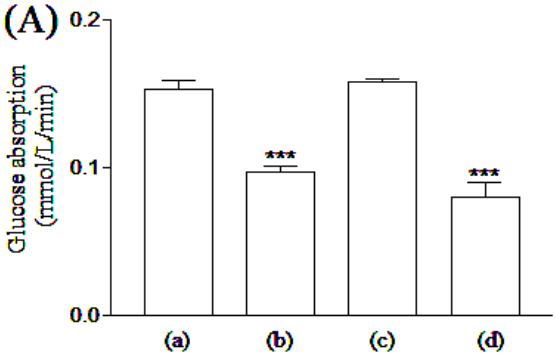


Figure 5A: Effect of catechin on intestinal glucose absorption in rat. Measured after 30 min; 10 mM Glucose (a); 10 mM glucose plus 1 mM phlorizin (b), 1 mM catechin (c), 1 mM phlorizin plus 1 mM catechin (d). Values are means ± S.E.M.; n=4 animals in each group. ***p< 0.001 compared with control group (a)

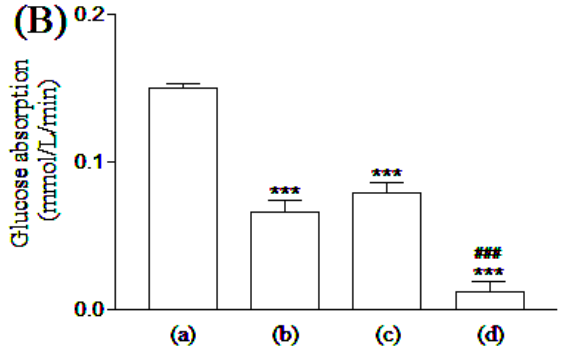


Figure 5B: Effect of catechin on intestinal glucose absorption in rat. Measured after 30 min; 10 mM Glucose (a); 10 mM glucose plus 1 mM phlorizin (b), 10 mM catechin (c), 1 mM phlorizin plus 10 mM catechin (d). Values are means ± S.E.M.; n=4 animals in each group. ***p<0.001 compared with control group (a), ###p<0.001 in relation to phlorizin group

The structural diversity of polyphenols makes the estimation of their content in food difficult. However, attempts to estimate the polyphenol intakes have been reported [29-31]. The mean and median of polyphenol intakes for the whole population is around 1.2 and 1.12 g/d [32] and the concentration in plasma rarely exceeds 1 μM after the consumption of 10–100 mg of a single phenolic compound [29].

From our results, myricitrin seems to be a powerful inhibitor of SGLT1 activity and also has alternative sites of action in intestine. Interestingly, these results are additive with antidiabetic effect of myricitrin by acting on aldose reductase and on glucose uptake in C2C12 myotubules under normal and insulin-stimulated conditions [33], suggesting a relevant role of myricitrin on glycemia homeostasis.

In our experimental conditions using 10mM of glucose, phlorizin effectively blocked the glucose absorption indicating that SGLT1 is inhibited. The following experiments were based on the influence of phlorizin (a specific inhibitor of SGLT1) on glucose absorption in the absence (control) or presence of some selected compounds. The inhibitory effect of phlorizin on intestinal glucose absorption was corroborated with those reported in the literature [23,27].

Although the evaluation of polyphenol dietary intake still lacks precision, the dietary intake of naringenin are reported to be about 0.2-0.5 for women and 0.18-0.55 g/d [39]. In our *in situ* approach studied, naringenin (both concentration assayed), significantly reduced intestinal glucose absorption by around 74% compared with glucose control group and similar to that observed for phlorizin. Furthermore, with both compounds together, the glucose absorption

almost disappeared suggesting a competition between them with the site of SGLT1.

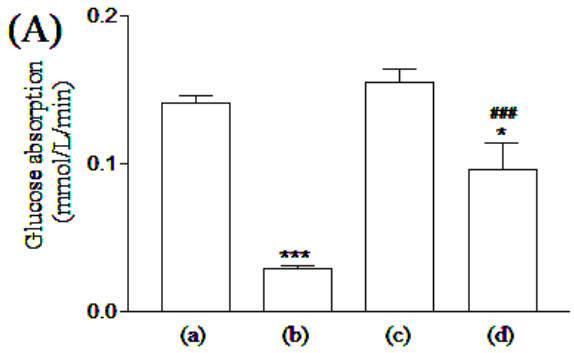


Figure 6A: Effect of naringenin on intestinal glucose absorption in rat. Measured after 30 min; 10 mM Glucose (a); 10 mM glucose plus 1 mM phlorizin (b), 1 mM naringenin (c), 1 mM phlorizin plus 1 mM naringenin (d). Values are means ± S.E.M.; n=4 animals in each group. *p< 0.05 and ***p< 0.001 compared with control group (a), ###p< 0.001 in relation to phlorizin group

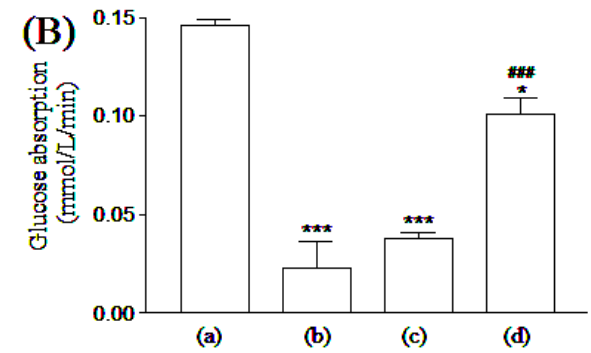


Figure 6B: Effect of naringenin on intestinal glucose absorption in rat. Measured after 30 min; 10 mM Glucose (a); 10 mM glucose plus 1 mM phlorizin (b), 10 mM naringenin (c), 1 mM phlorizin plus 10 mM naringenin (d). Values are means ± S.E.M.; n=4 animals in each group. *p<0.05, and ***p<0.001 compared with control group (a), ###p<0.001 in relation to phlorizin group

These data are in line with those reported for naringenin in rabbit and rat intestinal Brush Border Membrane Vesicle (BBMV) when compared with phlorizin. Additionally, the inhibitory effect of naringenin on glucose absorption compared with the powerful action of phorizin is in accordance with those reported for *per se* inhibition of naringenin on glucose absorption reported by Li et al. [27].

Quercetin, the main flavonol in our diet (present 0.3 mg/g in onions and 10-25 mg/L in tea) [34,35], showed an *in situ* inhibition as good as phlorizin on glucose absorption. These data are in agreement with those reported to quercetin on serum glucose lowering in hyperglycemic rats when this compound was administered by oral gavage [15]. Also for quercetin, it is reported that quercetin-3-O-glucoside did not affect SGLT1 and aglycone quercetin regulates glucose absorption by inhibition of GLUT-2 dependent uptake [36].

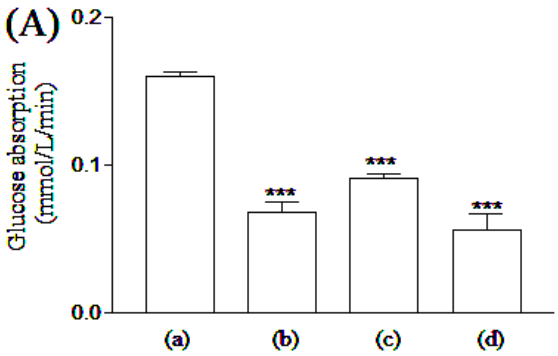


Figure 7A: Effect of caffeic acid on intestinal glucose absorption in rat. Measured after 30 min;10 mM Glucose (a); 10 mM glucose plus 1 mM phlorizin (b), 1 mM caffeic acid (c), 1 mM phlorizin plus 1 mM caffeic acid (d). Values are means ± S.E.M.; n=4 animals in each group. ***p< 0.001 compared with control group (a).

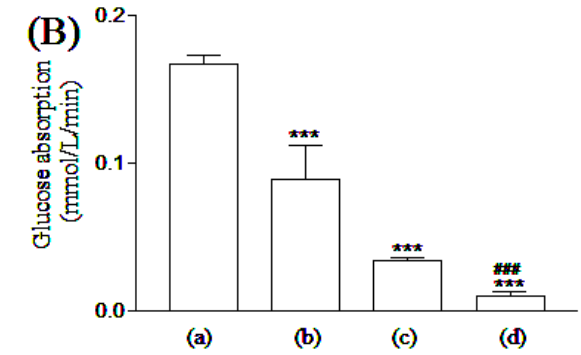


Figure 7B: Effect of caffeic acid on intestinal glucose absorption in rat. Measured after 30 min;10 mM Glucose (a); 10 mM glucose plus 1 mM phlorizin (b), 10 mM caffeic acid (c), 1 mM phlorizin plus 10 mM caffeic acid (d). Values are means ± S.E.M.; n=4 animals in each group. ***p<0.001 compared with control group (a), ###p<0.001 in relation to phlorizin group

One of the most abundant flavanol (1g/L) present in the infusion of green tea [37], catechin, beyond inhibit significantly the glucose uptake also potentiated the inhibitory effect of phlorizin on intestinal SGLT1 transporter and also catechin seems inhibit other hexoses transporters since its effect was greater than per se action of phlorizin. As described by Kobayashi et al. [38], catechin (1 mM) did not show significant inhibition on SGLT1. However, the potentiated effect of phlozin by catechin seems to be mediated by other targets since it was reported that the catechin structure increased the accessibility for another polyphenols residues to the glucose/Na^+-binding site in SGLT1 [38].

The most frequently phenolic acids encountered in foods are caffeic acid and also is found in the form of esters as chlorogenic acid. Caffeic acid [3,4-di(OH)-cinnamate], found in many types of fruit and coffee in high concentrations [29], exhibited an effective action on reduction of glucose uptake in intestine.

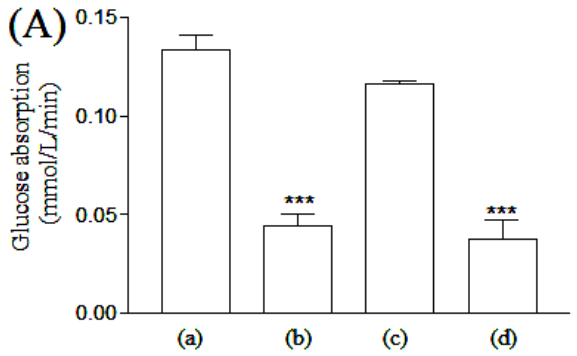


Figure 8A: Effect of rutin on intestinal glucose absorption in rat. Measured after 30 min; 10 mM Glucose (a); 10 mM glucose plus 1 mM phlorizin (b), 1 mM rutin (c), 1 mM phlorizin plus 1 mM rutin (d). Values are means ± S.E.M.; n=4 animals in each group. ***p< 0.001 compared with control group (a)

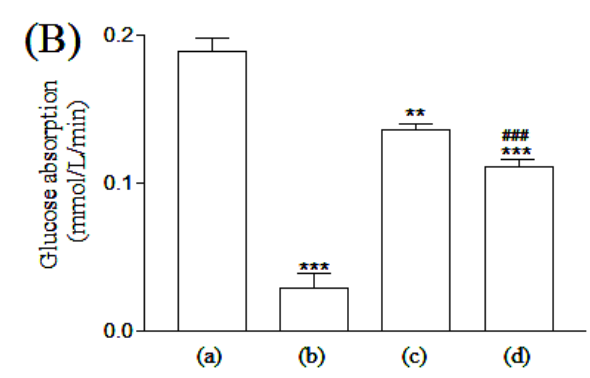


Figure 8B: Effect of rutin on intestinal glucose absorption in rat. Measured after 30 min; 10 mM Glucose (a); 10 mM glucose plus 1 mM phlorizin (b), 10 mM rutin (c), 1 mM phlorizin plus 10 mM rutin (d). Values are means ± S.E.M.; n = 4 animals in each group. **P< 0.01 and ***p< 0.001 compared with control group (a), ###p<0.001 in relation to phlorizin group

The role of caffeic acid on diabetes is reported in the literature by attenuating hepatic glucose output, increasing glucose uptake in adipocytes by stimulating insulin secretion. In a whole, caffeic acid suppresses the diabetes states [40].

Furthermore, we observed a similar profile of caffeic acid, myricitrin and catechin since both compounds potentiated the inhibitory effect of phlorizin on glucose uptake. Taking these results together, SGLT1 seems to be a target for some compounds that can be physiologically important to regulate glucose homeostasis during food intake.

It was observed for rutin and fukugetin a slight competitive effect with phlorizin on glucose uptake since both of them inhibited the phorizin effect. Rutin is known to decrease glycemia by acting in multiple tissues involved on glucose homeostasis [15,41,42]. Taking these data together, it reinforces the effective role of rutin as a candidate to possible application in controlling hyperglycemia and warrants further investigations. As far as we know, for fukugetin it is the first report concerning intestinal SGLT1 transporter as a target for this flavonoid.

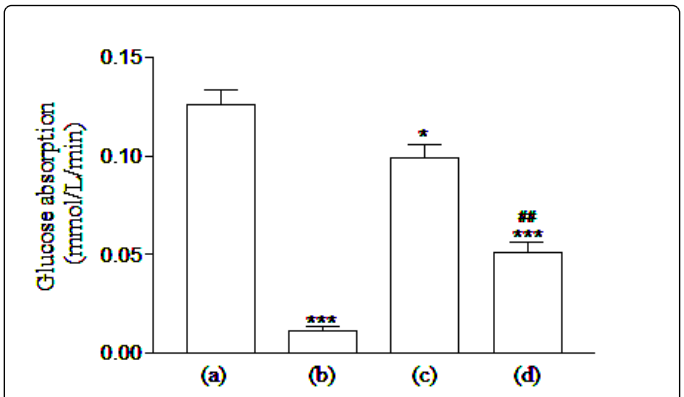


Figure 9: Effect of fukugetin on intestinal glucose absorption in rat. Measured after 30 min. 10 mM Glucose (a); 10 mM glucose plus 1 mM phlorizin (b), 10 mM fukugetin (c), 1 mM phlorizin plus 10 mM fukugetin (d). Values are means ± S.E.M.; n=4 animals in each group. *p<0.05 and ***p<0.001 compared with control group (a), ##p<0.01 in relation to phlorizin group

On the other hand, kaempferitrin already described by our group as antihyperglycemic agent by reducing serum glucose levels [15], in this *in situ* approach it does not changed glucose uptake. Also, hispidulin and chlorogenic acid also did not affect the SGLT1 activity. So, it is clear that the intestinal glucose transporters are not the main target for these compounds to regulate glucose homeostasis (data not shown). Other studies suggest that chlorogenic acid presents a hypoglycemic effect and might have an antagonistic effect on glucose transport [43,25]. However, for hispidulin only a report concerned its antidiabetic effect is available in the literature and the details concerned the mechanism of action is not available [44].

Conclusions

Taking together these data, we conclude that some selective nutraceutical compounds as myricitrin, quercetin, catechin, naringenin, caffeic acid, rutin and fukugetin are able to regulate glucose absorption by acting in an intestinal target, SGLT1, measured by *in situ* approach. In a whole, these data indicates that such compounds bind to the glucose transporter and antagonize the glucose transport similar to phlorizin. Studies are underway in order to elucidate the presence of these compounds also on sodium ionic balance in order to inhibit glucose absorption in intestine.

Acknowledgments

This work was supported by government grants by Conselho Nacional de Desenvolvimento e Tecnológico (CNPq), Coordenação de Pessoal de Nível Superior (CAPES)/PPG-Farmácia, and Fundação de

Amparo a Pesquisa do Estado de Santa Catarina (FAPESC). BGP is the recipient of a Master fellowship from CAPES-PPG-FARMÁCIA/ UFSC, Brazil.

References

1. Wright EM, Martín MG, Turk E (2003) Intestinal absorption in health and disease--sugars. Best Pract Res ClinGastroenterol 17: 943-956.
2. Silva FRMB, Frederico MJS, Castro AJG, Da Luz G, Altenhofen D, et al. (2013) Glucose uptake: knowledge from in vivo, in situ and in vitro studies and health implications. Glucose Uptake: Regulation, Signaling Pathways & Health Implications. Nova Science Publishers, New York.
3. Kellett GL, Brot-Laroche E (2005) Apical GLUT2: a major pathway of intestinal sugar absorption. Diabetes 54: 3056-3062.
4. Wright EM, Loo DD, Hirayama BA (2011) Biology of human sodium glucose transporters. Physiol Rev 91: 733-794.
5. Wright EM, Loo DD, Hirayama BA, Turk E (2004) Surprising versatility of Na+-glucose cotransporters: SLC5. Physiology (Bethesda) 19: 370-376.
6. Johnston K, Sharp P, Clifford M, Morgan L (2005) Dietary polyphenols decrease glucose uptake by human intestinal Caco-2 cells. FEBS Lett 579: 1653-1657.
7. Ehrenkranz JR, Lewis NG, Kahn CR, Roth J (2005) Phlorizin: a review. Diabetes Metab Res Rev 21: 31-38.
8. Drozdowski LA, Thomson AB (2006) Intestinal sugar transport. World J Gastroenterol 12: 1657-1670.
9. Cazarolli LH, Zanatta L, Alberton EH, Figueiredo MS, Folador P, et al. (2008) Flavonoids: prospective drug candidates. Mini Rev Med Chem 8: 1429-1440.
10. Pandey KB, Rizvi SI (2009) Plant polyphenols as dietary antioxidants in human health and disease. Oxid Med Cell Longev 2: 270-278.
11. Silva FRMB, Zanatta L, Frederico MJS, Pizzolatti MG, Campos AM (2013) Kaempferol and Kaempferitrin: Nutraceutical compounds contribute to glucose homeostasis by acting at multiple biological sites. Nova Science Publishers, New York.
12. Bahadoran Z, Mirmiran P, Azizi F (2013) Dietary polyphenols as potential nutraceuticals in management of diabetes: a review. J Diabetes MetabDisord 12: 43.
13. Cazarolli LH, Zanatta L, Jorge AP, de Sousa E, Horst H, et al. (2006) Follow-up studies on glycosylated flavonoids and their complexes with vanadium: their anti-hyperglycemic potential role in diabetes. ChemBiol Interact 163: 177-191.
14. Cazarolli LH, Pereira DF, Kappel VD, Folador P, FigueiredoMdos S, et al. (2013) Insulin signaling: a potential signaling pathway for the stimulatory effect of kaempferitrin on glucose uptake in skeletal muscle. Eur J Pharmacol 712: 1-7.
15. Pereira DF, Cazarolli LH, Lavado C, Mengatto V, Figueiredo MS, et al. (2011) Effects of flavonoids on Î±-glucosidase activity: potential targets for glucose homeostasis. Nutrition 27: 1161-1167.
16. Pereira DF, Kappel VD, Cazarolli LH, Boligon AA, Athayde ML, et al. (2012) Influence of the traditional Brazilian drink Ilex paraguariensis tea on glucose homeostasis. Phytomedicine 19: 868-877.
17. Pizzolatti MG, Junior Cunha A, Szpoganicz B, De Sousa E, Filho RB, et al. (2003) Flavonóidesglicosilados das folhas e flores de Bauhinia forficata (Leguminosae). Quim Nova 26: 466-469.
18. Moreira FPM, Coutinho V, Montanher ABP, Caro MSB, Brighente IMC, et al. (2003) Flavonóides e triterpenos de Baccharispseudotenuifolia – bioatividadesobreArtemiasalina. Quim Nova 26: 309-311.
19. Nardi GM, Dalbó S, Monache FD, Pizzolatti MG, Ribeiro-do-Valle RM (2006) Antinociceptive effect of Croton celtidifoliusBaill (Euphorbiaceae). J Ethnopharmacol 107: 73-78.
20. Verdi LG, Pizzolatti MG, Montanher AB, Brighente IM, SmâniaJúnior A, et al. (2004) Antibacterial and brine shrimp lethality tests of biflavonoids and derivatives of Rheediagardneriana. Fitoterapia 75: 360-363.
21. LapaFda R, Gadotti VM, Missau FC, Pizzolatti MG, Marques MCA, et al. (2009) Antinociceptive properties of the hydroalcoholic extract and the flavonoid rutin obtained from Polygala paniculata L. in Mice. Basic ClinPharmacolToxicol 104: 306-315.
22. Meotti FC, Missau FC, Ferreira J, Pizzolatti MG, Mizuzaki C, et al. (2006) Anti-allodynic property of flavonoid myricitrin in models of persistent inflamMatory and neuropathic pain in mice. BiochemPharmacol 72: 1707-1713.
23. Gonzalez-Mujica F, Motta N, Márquez AH, Capote-Zulueta J (2003) Effects of Bauhinia megalandra aqueous leaf extract on intestinal glucose absorption and uptake by enterocyte brush border membrane vesicles. Fitoterapia 74: 84-90.
24. Varley H, Gowenlock AH, Bell M (1976) Practical Clinical Biochemistry. In: Heinemann IW, (5thedn), Medical Books Ltd, London, UK.
25. Nicasio P, Aguilar-Santamaría L, Aranda E, Ortiz S, González M (2005) Hypoglycemic effect and chlorogenic acid content in two Cecropia species. Phytother Res 19: 661-664.
26. Kellett GL, Brot-Laroche E, Mace OJ, Leturque A (2008) Sugar absorption in the intestine: the role of GLUT2. Annu Rev Nutr 28: 35-54.
27. Li JM, Che CT, Lau CB, Leung PS, Cheng CH (2006) Inhibition of intestinal and renal Na+-glucose cotransporter by naringenin. Int J Biochem Cell Biol 38: 985-995.
28. MANOME S, KURIAKI K (1961) Effect of insulin, phlorizin and some metabolic inhibitors on the glucose absorption from the intestine. Arch IntPharmacodynTher 130: 187-194.
29. Scalbert A, Williamson G (2000) Dietary intake and bioavailability of polyphenols. J Nutr 130: 2073S-85S.
30. Pérez-Jiménez J, Fezeu L, Touvier M, Arnault N, Manach C, et al. (2011) Dietary intake of 337 polyphenols in French adults. Am J ClinNutr 93: 1220-1228.
31. González S, Fernández M, Cuervo A, Lasheras C (2014) Dietary intake of polyphenols and major food sources in an institutionalised elderly population. J Hum Nutr Diet 27: 176-183.
32. Pérez-Jiménez J, Neveu V, Vos F, Scalbert A (2010) Systematic analysis of the content of 502 polyphenols in 452 foods and beverages: an application of the phenol-explorer database. J Agric Food Chem 58: 4959-4969.
33. Ding Y, Dai XQ, Zhang ZF, Li Y (2012) Myricetin attenuates hyperinsulinemia-induced insulin resistance in skeletal muscle cells. Eur Food Res Technol 234: 873-881.
34. Hertog MGL, Hollman PCH, Katan MB (1992) Content of potentially anticarcinogenic flavonoids of 28 vegetables and 9 fruits comMonly consumed in the Netherlands. J Agric Food Chem 40: 2379-2383.
35. Hertog MGL, Hollman PCH, Van de Putte B (1993) Content of potentially anticarcinogenic flavonoids of tea infusions, wine and fruit juices. J Agric Food Chem 41: 1242-1246.
36. Williamson G (2013) Possible effects of dietary polyphenols on sugar absorption and digestion. MolNutr Food Res 57: 48-57.
37. Lee MJ, Wang ZY, Li H, Chen L, Sun Y, et al. (1995) Analysis of plasma and urinary tea polyphenols in human subjects. Cancer Epidemiol Biomarkers Prev 4: 393-399.
38. Kobayashi Y, Suzuki M, Satsu H, Arai S, Hara Y, et al. (2000) Green tea polyphenols inhibit the sodium-dependent glucose transporter of intestinal epithelial cells by a competitive mechanism. J Agric Food Chem 48: 5618-5623.
39. Koch W, Kukula-Koch W, Marzec Z, Marc D (2013) Application of TLC method with video scanning in estimation of daily dietary intake of specific flavonoids--preliminary studies. Acta Pol Pharm 70: 611-620.
40. Jung UJ, Lee MK, Park YB, Jeon SM, Choi MS (2006) Antihyperglycemic and antioxidant properties of caffeic acid in db/db mice. J PharmacolExpTher 318: 476-483.
41. Kappel VD, Zanatta L, Postal BG, Silva FR (2013) Rutin potentiates calcium uptake via voltage-dependent calcium channel associated with stimulation of glucose uptake in skeletal muscle. Arch BiochemBiophys 532: 55-60.
42. Kappel VD, Frederico MJ, Postal BG, Mendes CP, Cazarolli LH, et al. (2013) The role of calcium in intracellular pathways of rutin in rat

pancreatic islets: potential insulin secretagogue effect. Eur J Pharmacol 702: 264-268.

43. Johnston KL, Clifford MN, Morgan LM (2003) Coffee acutely modifies gastrointestinal hormone secretion and glucose tolerance in humans: glycemic effects of chlorogenic acid and caffeine. Am J ClinNutr 78: 728-733.

44. Feihua Wu, Jingyu Liang, Hui Wang, Weiguang Li (2011) Flavonoid compound for preventing and treating diabetes and medicament application thereof. China patent CN 102151281 A.