Flavonoids: Cellular and Molecular Mechanism of Action in Glucose Homeostasis

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Abstract: The purpose of this review is to discuss the cellular and molecular mechanisms of action of flavonoids focusing on carbohydrate metabolism. The beneficial effects of flavonoids have been studied in relation to diabetes mellitus, either through their capacity to avoid glucose absorption or to improve glucose tolerance. Furthermore, flavonoids stimulate glucose uptake in peripheral tissues, regulate the activity and/or expression of the rate-limiting enzymes in the carbohydrate metabolism pathway and act *per se* as insulin secretagogues or insulin mimetics, probably, by influencing the pleiotropic mechanisms of insulin signaling, to ameliorate the diabetes status.

Key Words: Flavonoids, mechanism of action, glycemia, insulin, diabetes.

BIOLOGICAL EFFECTS OF FLAVONOIDS

In the physiological state, the maintenance of glucose homeostasis is achieved by a hormonal regulation of glucose uptake and endogenous glucose production by the muscle and liver, respectively. Diabetes mellitus is a complex metabolic disorder in the endocrine system characterized by abnormalities in insulin secretion and/or insulin action that lead to progressive deterioration of glucose tolerance and cause hyperglycemia. This disease is a major public health problem found in all parts of the world and is rapidly increasing [1]. There are basically two types of diabetes: a) type 1, insulin-dependent diabetes mellitus (IDDM), in which the body does not produce insulin, most often occurs in children and young adults; b) type 2, noninsulin-dependent diabetes mellitus (NIDDM), characterized by insulin-resistance due to an improper use of insulin. It is the most common form of the disease, occurring mainly in elderly people [1].

Recently, there has been a growing interest in hypoglycemic agents from natural products, especially those derived from plants. Flavonoids are naturally occurring phenolic compounds that are widely distributed in plants. They have a broad range of biological activities and numerous studies have been carried out on their potential role in the treatment of diabetes. Many studies have demonstrated the hypoglycemic effects of flavonoids using different experimental models and treatments. Intraperitoneal injection of epigallocatechin gallate into rats has been found to lowered blood glucose and insulin levels [2]. In the same way, green tea has been observed to improve glucose metabolism in healthy humans in oral glucose tolerance tests. Green tea also lowered blood glucose levels in diabetic db/db mice and streptozotocin-diabetic (STZ-diabetic) mice 2–6 h after administration without affecting the serum insulin level [3]. Administration of an aqueous solution of green tea in normal and alloxan-diabetic rats improved glucose tolerance and reduced blood glucose levels, respectively [4]. Furthermore, studies with procyanidins administered alone have demonstrated a significant reduction in glycemic levels and together with insulin they showed an addictive hypoglycemic effect in rats [5].

Bolus intravenous injection of puerarin, an isoflavonoid, has been reported to decrease the plasma glucose concentrations in STZ-diabetic, normal and hyperglycemic rats [6]. In addition, glucose tolerance tests with puerarin showed a significant blunting of the rise in blood glucose compared with control C57BL/6J-ob/ob mice [7]. Another isoflavonoid, genistein, has been shown to significantly decrease the blood glucose level in diabetic rats compared with the control, in glucose tolerance tests. These results were confirmed with genistein and daidzein chronic treatments in db/db mice and STZ-diabetic rats [8, 9].

Studies have been carried out on the effects of kaempferitrin, one of the compounds found in the *n*-butanol fraction of *Bauhinia forficata*, on glycemia in diabetic rats. The hypoglycemic effect of kaempferitrin in diabetic rats was evident at all doses tested and this profile was maintained throughout the study period. Additionally, in glucose-fed hyperglycemic normal rats, the kaempferitrin failed to decrease blood glucose levels [10]. Taking advantage of the hypoglycemic effect of vanadium, a vanadium-based flavonoid complex has been designed and studied. Kaempferitrin–VO(IV) (vanadium complex) as well as VO(IV) in alloxan-diabetic rats demonstrated hypoglycemic effect for 1 to 24 h when compared to the respective zero time. The kaempferol-3-neohesperidoside, a glycosylated flavonoid structurally very similar to kaempferitrin has shown an interesting hypoglycemic effect in both oral and intraperitoneal treatments in

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1389-5575/08 $55.00+.00

diabetic rats. When complexed with vanadium, the kaempferol-3-neohesperidoside–VO (IV) exhibited a powerful hypoglycemic effect throughout the post-treatment period studied when compared to zero time [11]. When complexed with vanadium, quercetin, exhibited highly potent insulin-enhancing activity in STZ-diabetic mice with no effect on the blood glucose level of normal mice, in agreement with the results for kaempferitrin and kaempferol-3-neohesperidoside-VO complexes [11, 12].

The myricetin flavonol, when injected intravenously into rats, attenuated the increase of plasma glucose levels after an intravenous glucose challenge [13]. Also, the treatment of diabetic rats with myricetin resulted in the lowering of glycemia by 50% after 2 days. Myricetin, however, did not have an effect on the serum glucose levels in normal rats [14]. Another flavonol extensively studied in relation to its potential role in diabetes is quercetin. Vessal *et al.* [15] demonstrated that quercetin reduced the blood glucose level of diabetic rats in 8–10 days of treatment. In the same study, quercetin exerted no effect on the glucose tolerance curve either in normoglycemic or in STZ-diabetic rats. These results are in agreement with those described by Shetty *et al.* [16] for hypoglycemic effects of quercetin in diabetic rats.

Anti-hyperglycemic and hypoglycemic effects have been demonstrated for various flavonoids including chrysin and its derivatives, silymarin, isoquercetrin and rutin [17-19]. Long-term studies carried out with rutin orally administered to diabetic rats showed that it decreased the plasma glucose levels by up to 60% when compared to the control group. However, oral administration of rutin to normal rats did not show any significant effect on fasting plasma glucose levels [20]. Chronic treatment with hesperidin and naringin was found to lower the blood glucose level of db/db mice compared with the control group [21]. Intraperitoneal administration of prunin (naringenin 7-O-β-D-glucoside), a glycoside from naringenin, produced a significant hypoglycemic effect in diabetic rats [22].

Several studies have demonstrated that flavonoids are absorbed in the intestine and in some cases they compete with glucose in certain absorption mechanisms. A reduction in the intestinal absorption of glucose constitutes a possible means of controlling diabetic hyperglycemia. *In vitro* studies have shown that a soybean extract containing the isoflavones genistein and daidzein inhibits glucose absorption into the intestinal brush border membrane vesicles of rabbits [23]. Naringenin, a flavonoid present in citrus fruits and juices, inhibited glucose absorption in the intestine. Naringenin showed strong inhibitory action in rat everted intestinal sleeves in a competitive manner. In the same study, the authors observed that naringenin reduced glucose uptake in the intestinal brush border membrane vesicles of diabetic rats to a level similar to that of normal rats [24]. (-)Epicatechin gallate [25], myricetin, quercetin, apigenin, (-) epigallocatechin gallate, and (-)-epigallocatechin demonstrated a marked reduction in glucose absorption, when compared with the control, by competitive inhibition of sodium-dependent glucose transporter-1. The non-glycosylated polyphenols have been shown to reduce glucose absorption under sodium-dependent conditions *in vivo* and *in vitro* in animal tissues [26, 27].

Besides reducing glucose absorption, another possible mechanism to control blood glucose levels is the inhibition of α-glucosidase activity in the intestine. Cyanidin-3-α-O-rhamnoside and pelargonidin-3-α-O-rhamnoside, two anthocyanins, have been observed to exhibit inhibitory effects on glucose absorption and on α-glucosidase activity *in vitro* by CaCo2 cells [28]. Inhibitory effects on α-glucosidase activity were demonstrated when luteolin, kaempferol, chrysin and galangin were used both *in vitro* and *in vivo* to study the potential role in the absorption and metabolism of carbohydrates [29]. The α-glucosidase inhibitory activity of flavonoids was confirmed in a study by Kim *et al.* [30], where it was shown that luteolin, amentoflavone, luteolin 7-O-glucoside and daidzein were the strongest inhibitors of the compounds tested.

Under physiological conditions, the reabsorption of glucose from renal filtrate to plasma is determined primarily by sodium-coupled glucose transporters located on the luminal membrane of the proximal tubule of the kidney. This represents important contribution to glucose homeostasis. Glucose present in urine is one of the symptoms of diabetic patients and can cause serious complications such as nephropathy. To revert this renal complication, some flavonoids have been studied due to their effect on renal glucose reabsorption and excretion. Human treatment with sylimarin can decrease glucosuria and glycemia after four months of daily ingestion of the flavonoid [18]. Naringenin has been shown to inhibit the glucose reabsorption in renal brush border membrane vesicles when compared to normal rats, while naringin was not found to have an effect [24]. In a study on quercetin, diabetic and normal rats were submitted to a long-term treatment with quercetin to evaluate urinary parameters. The quercetin-fed diabetic group showed an improvement in the polyurea state and the excretion of urine was lower throughout the study period when compared with the control groups. In addition, a quercetin-fed diabetic group demonstrated an improvement in the amount of sugar excreted in urine [16]. For kaempferitrin, no alteration in urinary glucose levels was detected in the urine of normal and diabetic rats collected for 3 h after the oral treatment [31]. Recently, flavonoids have been reported to affect renal advanced glycation end-products and protein expression, which are involved in diabetic nephropathy, for example, puerarin [32]. (-)-Epigallocatechin 3-O-gallate administration over a 50 day-period to diabetic rats has shown suppressed hyperglycemia, proteinuria and reduced renal advanced glycation end-product accumulation and its related protein expression in the kidney and pathological conditions associated with nephropathy [33]. Green tea flavonoids can attenuate urinary protein excretion and the morphological changes particular to diabetic nephropathy after long-term treatment. Also hyperglycemia, as assessed in terms of blood glucose and glycosylated protein levels, can be improved by administration of green tea flavonoids in diabetic rats [34].

MOLECULAR MECHANISM OF FLAVONOIDS COMPARED WITH INSULIN SIGNAL TRANSDUCTION

Insulin is the most important hormone in the regulation of blood glucose concentrations and is essential in the postprandial state. When blood sugar concentrations rise, insulin

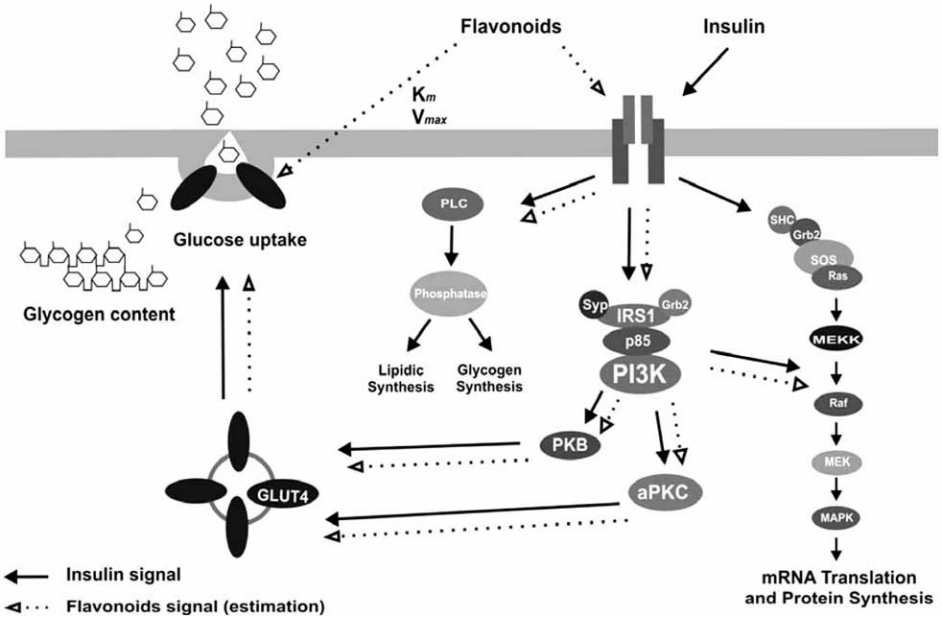

Fig. (1). Insulin binds to the insulin receptor (IR), thereby activating the intrinsic kinase activity in the β-subunit, which results in autophosphorylation and recruitment of substrates, such as insulin receptor substrate (IRS1-4) proteins, Cbl and SHC. Phosphorylated IRS proteins provide docking sites for proteins with Src Homology 2 (SH2) domains. Many of these proteins are adaptor molecules such as the regulatory subunit of PI3K, or the adaptor molecule Grb2, which associates with SOS to activate the Ras–MAPK pathway. The PI3K enzyme consists of regulatory (p85) and catalytic (p110) subunits which catalyze the formation of the lipid second messenger PIP3 in the cell, an allosteric activator of phosphoinositide-dependent kinase (PDKs). Targets of PDK include PKB/Akt and the atypical protein kinase C (PKC) isoforms. Together with PI3K, activated PKB/Akt and atypical PKCs are involved in the insulin-stimulated GLUT4 translocation, glucose uptake and glycogen synthesis.

is secreted into the blood stream by β-cells of the endocrine pancreas and glucose is the primary stimulus for insulin secretion [35]. Initially, glucose enters β-cells through the high capacity glucose transporter type 2 (GLUT 2) and is phosphorylated by glucokinase. The generation of ATP from glycolysis increases the intracellular ATP/ADP ratio [36]. ATP binds to ATP-dependent K^+-channels on the β-cell membranes closing these channels and depolarizing the cells. The depolarization activates voltage-sensitive calcium channels causing a calcium influx triggering insulin secretion [37].

Researchers have proposed flavonoids as potential antidiabetic agents since they exert multiple actions on the synthesis and release of insulin from β-cells. The supplementation of genistein increases the plasma insulin of the STZ-diabetic rats [8]. *In vitro* studies have shown that genistein can increase insulin secretion from mouse pancreatic islets in the presence of glucose. Consistent with this effect, genistein increases intracellular cAMP, probably by enhancing adenylate clyclase activity and activating protein kinase A (PKA) by a mechanism that does not involve protein tyrosine kinase **(**PTK). These findings demonstrate that genistein directly acts on pancreatic β-cells, leading to activation of the cAMP/PKA signaling cascade to exert an insulinotropic effect [38].

Oral administration of rutin in the long-term treatment of diabetic rats has been found to significantly increase plasma insulin and C-peptide levels. A histopathological study of the pancreas revealed the protective role of rutin resulting in β-cell proliferation [39]. An increase in the number of pancreatic islets has also been observed in both normoglycemic and diabetic rats treated with quercetin. This effect may be due to increased DNA replication of β-cells [15, 40]. Jayaprakasam *et al.* [41] characterized the effect of cyanidin-3-glucoside and delphinidin-3-glucoside as one of the most effective insulin secretagogues of the anthocyanins and anthocyanidins tested. Also, pelargonidin-3-galactoside and its aglycone, pelargonidin, can cause a significant increase in insulin secretion in the presence of glucose.

Once insulin is released by β-cells the molecular signaling is mediated by a complex mechanism of action. In the presence of hormones, the activated insulin receptor phosphorylates the insulin receptor substrate proteins (IRS proteins), which are linked to the activation of signaling pathways: the phosphatidylinositol 3-kinase (PI3K)–AKT/protein kinase B (PKB) pathway, which is responsible for most of the metabolic actions of insulin; and the Ras–mitogen-activated protein kinase (MAPK) pathway, which regulates expression of some genes and cooperates with the PI3K pathway to control cell growth and differentiation [42]. Alternatively, through specific phospholipase C activity, a second messenger (inositol phosphate glycan/IPG) can be produced which activates the protein phosphatases which in turn regulate glucose and lipid metabolism [43] (Fig. (**1**)).

Cellular glucose transport is mediated through solute carriers referred to as the family of facilitative glucose transporters (GLUTs), each one with different tissue distributions,

kinetic properties and sugar specificity. The GLUT-1 transporter is ubiquitously expressed and responsible for basal glucose uptake. The GLUT-2 isoform is primarily expressed in β-cells and in the liver, and has a relatively low affinity (high K_m) for glucose that in combination with hexokinase serves as part of the glucose sensor. GLUT-3 has the highest affinity (lowest K_m) and is expressed during fetal development and in adult neurons. Similar to the tightly controlled distribution and functionality of the other GLUT family members, GLUT-4 is predominantly restricted to fat and muscle and is responsible for insulin-stimulated glucose uptake. This process occurs through a complex and, as yet, incompletely defined signaling pathway involving the insulin receptor tyrosine kinase. The primary effect is to promote the transport of GLUT-4 from intracellular storage sites to the plasma membrane. In the basal state, GLUT-4 is localized in intracellular vesicles, while in the presence of insulin GLUT-4 is immunolocalized in the plasma membrane of fat, skeletal and cardiac muscle. The rate-limiting step at which insulin stimulates uptake of glucose in muscle and fat is the translocation of GLUT-4 transporters to the plasma membrane [43, 44].

This complex system of insulin-stimulated whole-body glucose utilization is impaired in people with diabetes. The molecular defects accounting for impaired glucose utilization are not fully understood but may involve defective GLUT4 translocation, glucose uptake and aberrant insulin signal transduction. In this context, several naturally occurring polyphenols have been shown to affect glucose transport and insulin-receptor function, both of which play essential roles in diabetes.

In vitro experiments with the rat diaphragm have shown that luteolin 5-rutinoside can increase glucose uptake and insulin secretion suggesting that this flavonoid can act as an anti-hyperglycemic and hypoglycemic agent [45]. In rat soleus muscle, kaempferitrin stimulated significantly the glucose uptake compared to the control. Kaempferitrin reportedly has no effect on protein synthesis in muscle from normal and diabetic rats treated with this compound. Considering these results, it has been proposed that kaempferitrin acts as an insulin mimetic flavonoid [31].

Rat adipocytes incubated in the presence of myricetin have shown an increased rate of both D-glucose and D-3-O-methyl-glucose uptake without affecting insulin receptor autophosphorylation, tyrosine kinase activity of the receptor or glucose transporter translocation to the plasma membrane. It has been demonstrated that the stimulatory effect of myricetin on glucose uptake involves an increase in glucose transporter V_{max} values [14, 46]. This stimulatory effect of myricetin on glucose uptake has also been observed in the soleus muscle of diabetic rats [13]. Liu *et al.* [47] demonstrated that GLUT4 mRNA and protein levels in the soleus muscle of diabetic rats were lower in comparison to the normal control rats. Additionally, chronic treatment of diabetic rats with myricetin can result in an elevation of GLUT4 gene expression and protein levels.

Hesperidin or naringin treatments have resulted in a significant reduction in the hepatic GLUT2 expression in db/db mice, while the expression of adipocyte GLUT4 level increased [48]. Puerarin, an isoflavone, in the soleus muscle of diabetic rats also enhanced the uptake of glucose. Moreover, the mRNA and GLUT4 transporter protein in soleus muscle were observed to increase after repeated administration of puerarin in diabetic rats. Probably, one of the mechanisms of puerarin in glucose uptake in muscle is mediated by a GLUT4 gene expression increase [6, 7].

The hypoglycemic effects of green tea catechins have been confirmed both *in vivo* and *in vitro*. Green tea supplementation in chronic treatment stimulated the basal and insulin-stimulated glucose uptake in adipocytes as well as GLUT4 content [49]. The effects of epigallocatechin gallate and (-) epicatechin have also been demonstrated *in vitro* [50, 51].

The antihyperglycemic effect of procyanidins has been studied in two insulin-sensitive cell lines. Procyanidin treatment caused an increase in glucose uptake in cell lines, L6E9 myotubes and 3T3-L1 adipocytes. Similarly to the action of insulin, the effect of procyanidins on glucose uptake was sensitive to wortmannin, an inhibitor of PI3K, and to SB203580, an inhibitor of p38MAPK. Procyanidins also stimulated the GLUT4 translocation to the plasma membrane suggesting that they mimic and/or influence the insulin effect by directly acting on specific components of the insulin-signaling transduction pathway [5]. Similarly, it has been demonstrated for kaempferol 3-neohesperidoside that the stimulatory effect on glucose uptake in muscle is *via* the PI3K and PKC pathways and is, at least in part, independent of the MEK pathway and the synthesis of new glucose transporters [52] (Fig. (**1**)).

In contrast with several reports regarding the stimulatory effect of flavonoids on insulin signal transduction, GLUT4 translocation and glucose transport, some natural compounds act negatively on these pathways. For example, naringenin can inhibit insulin-stimulated glucose uptake in a dose-dependent manner in adipocytes. Naringenin has been reported not to alter the phosphotyrosine status of the insulin receptor, IRS proteins, or PI3K, however, it was found to inhibit the phosphorylation of the downstream signaling molecule Akt. In an *in vitro* PI3K assay, naringenin, like wortmannin, blocked the production of PIP3 by immunoprecipitated PI3K [53]. Also, genistein inhibited insulin-stimulated glucose uptake in 3T3-L1 adipocytes without affecting insulin-induced tyrosine kinase activity of the insulin receptor or activation of PKB. These results suggest that genistein can interfere with the insulin-induced glucose uptake directly and not by inhibiting GLUT4 translocation. This is in agreement with previous reports in the literature [54, 55].

In the same way, the flavonoids catechin-gallate, quercetin and myricetin can inhibit insulin-stimulated methylglucose uptake in rat adipocytes [56]. Moreover, evidence points to the fact that quercetin, myricetin and catechin-gallate inhibit glucose uptake due to a direct interaction with GLUT4, acting as competitive blockers of glucose transport [56, 57]. Interestingly, genistein, quercetin, apigenin and kaempferol have also been described as potent tyrosine kinase inhibitors [58].

The effect of insulin either on intermediate metabolism regulation or as growth factor is a consequence of the hor-

mone binding to the specific receptor at the plasma membrane in the target tissues of insulin. Post-receptor signal transduction can culminate with activated anabolic pathways as well as cell proliferation. After entering the hepatocytes or muscle cells, glucose is immediately phosphorylated by glucokinase (hexokinase IV) or hexokinase I and II in glucose-6-P. From glucose-6-phosphate, the glucose flux is directed to glycogen synthesis, glycolysis or to triglyceride synthesis [59]. Insulin inhibits gluconeogenesis and glycogenolysis through a phosphorylation mechanism and regulates the expression of genes encoding hepatic and muscular enzymes. This hormone inhibits the transcription of the gene encoding phosphoenolpyruvate carboxykinase, the rate-limiting step in gluconeogenesis [60]. Insulin also decreases transcription of the genes encoding fructose-1,6-biphosphatase and glucose-6-phosphatase (G-6-Pase), and increases transcription of glycolytic enzymes such as glucokinase and pyruvate kinase, and lipogenic enzymes such as fatty acid synthase and acetyl-CoA carboxylase [61, 62].

Glucose-6-phosphatase catalyses the dephosphorylation of glucose-6-phosphate in the liver and represents the ultimate step prior to the release of free glucose into the hepatic veins, be it derived from glycogenolysis or gluconeogenesis. As only the liver and the kidneys express glucose-6-phosphatase, only in these tissues can gluconeogenesis result in the release of free glucose to the blood stream [59].

Insulin acts directly in the accumulation and breakdown of glycogen in the liver and in the skeletal muscles. The hormone activates glycogen synthase by promoting its dephosphorylation, through the inhibition of kinases such as PKB or glycogen synthase kinase-3 (GSK-3) [63] and activation of protein phosphatase 1 (PP1) leading to increased glycogen synthesis kinase 3. On the other hand, insulin inhibits glycogenolysis, by dephosphorylating glycogen phosphorylase, through the activation of protein phosphatases [64, 65] (Fig. (**1**)).

In this regard, the supplementation of genistein to diabetic and normal rats was found to increase the glucokinase level in the diabetic rats. A significant reduction in G-6-Pase was observed in the groups treated with genistein [8]. The genistein and daidzein supplementation of diabetic (db/db) and non-diabetic mice was found to elevate hepatic glucokinase activity, while it suppressed hepatic G-6-Pase and phosphoenolpyruvate carboxykinase activity in db/db mice. Genistein and daidzein supplements enhanced hepatic glycogen in diabetic treated db/db mice when compared with diabetic db/db group and normal db/+ group [9].

It has recently been reported that epigallocatechin 3-gallate mimics the effects of insulin on the gene expression reduction of phosphoenolpyruvate carboxykinase and G-6-Pase in the mouse liver [66]. Like insulin, epigallocatechin 3-gallate increases tyrosine phosphorylation of the insulin receptor and insulin receptor substrate-1 (IRS-1), mitogen-activated protein kinase, p70s6k, and PI3K activity, and reduces phosphoenolpyruvate carboxykinase gene expression mediated by PI3K [67]. Furthermore, epigallocatechin 3-gallate upregulates glucokinase mRNA expression in the liver of db/db mice [68].

Hesperidin and naringin supplemented groups can increase hepatic glucokinase activity and glycogen concentration. Naringin has also been observed to markedly lower the activity of hepatic G-6-Pase and phosphoenolpyruvate carboxykinase. The results suggested that hesperidin and naringin improves hyperglycemia by regulating the activity of the hepatic enzymes involved in glycolysis and gluconeogenesis [21]. Hesperidin and naringin both significantly increase the glucokinase mRNA, while naringin also lowers the mRNA expression of phosphoenolpyruvate carboxykinase and G-6-Pase in the liver [48].

In another study, oral administration of rutin to diabetic rats resulted in a decrease in plasma glucose and increase in insulin levels, and restored the glycogen content and hexokinase activity. The activity of enzymes such as G-6-Pase and fructose-1,6-bisphosphatase significantly decreased in the liver and muscles of rutin-treated diabetic rats [39].

Intraperitoneal quercetin treatment has been found to increase hexokinase and glucokinase activity in diabetic rats without effecting normal rats [15]. Also, quercetin has demonstrated a potent inhibitory effect on both glycogen phosphorylase *a* (phosphorylated, active) and *b* (unphosphorylated, inactive) in isolated muscle [69]. Also, quercetin and other flavonols inhibited the rat liver G-6-Pase activity. The highest inhibitory activity was shown by quercetin 3-*O*-α-(2"-galloyl)rhamnoside and kaempferol 3-*O*-α-(2"-galloyl) rhamnoside. Quercetin 3-*O*- α-(2"-galloyl)rhamnoside and kaempferol 3-*O*- α-(2"-galloyl)rhamnoside exhibited the lowest IC_{50} of all the flavonoids assayed. Quercetin 3-*O*- α-(2"-galloyl)rhamnoside increased the K_m for glucose-6-phosphate without changes in the V_{max} and strongly inhibited the neoglucogenic capacity of rat liver. The G-6-Pase inhibition by quercetin 3-*O*- α-(2"-galloyl)rhamnoside might explain the decrease in the liver neoglucogenesis and, in turn, the reduction in glucose levels observed in diabetic patients [70, 71].

Diabetic and normal rats treated with myricetin have shown reduced hyperglycemia after 2 days of treatment. The treatment increased the hepatic glycogen and glucose-6-phosphate levels in diabetic rats. It also increased hepatic glycogen synthase I activity without having any effect on either total glycogen synthase or hepatic phosphorylase *a* activity, however, it lowered phosphorylase *a* activity in the muscle [14]. As with myricetin, catechin can cause an increase in hepatic glycogen and in glucose uptake in rats after *in vivo* treatment. In the same way, glycogen synthase activity increases significantly whereas glycogen phosphorylase decreases, which is consistent with glycogen storage in the liver [72].

There has been considerable scientific progress over the past few years in unraveling of the effect and mechanism of action of flavonoids. The major potential benefits of flavonoids reported over the past 15-20 years discussed in this review clearly demonstrate that these exogenous substances represent an unparalleled source of molecular diversity in relation to the drug discovery process. This is of great importance given that the molecular mechanism of action of insulin is well known. However, many gaps remain in our understanding of such processes, ranging from the absorption of

flavonoids in the enterocytes to cellular behavior changes. Furthermore, we still need to define the missing steps in the flavonoid-signaling network and elucidate the mechanism of cross-talk based on the complex mechanism of insulin action, in order to provide new insights into the potential role of flavonoids in diabetes treatment.

ACKNOWLEDGEMENTS

Studies in the authors' laboratory were supported by Conselho Nacional de Desenvolvimento e Tecnológico (CNPq); Coordenação de Pessoal de Nível Superior (CAPES) and Fundação de Amparo à Pesquisa do Estado de Santa Catarina (FAPESC) for the past 5 years. The authors express their appreciation to Dr. Siobhan Wiese for assistance with the English correction of the manuscript and to Leila Zanatta for the schematic drawings of the molecular mechanisms.

ABBREVIATIONS

IDDM	=	Insulin-dependent diabetes mellitus
NIDDM	=	Noninsulin-dependent diabetes mellitus
STZ	=	Streptozotocin
VO(IV)	=	Vanadium IV
GLUT	=	Glucose transporter
ATP	=	Adenosine 5'-triphosphate
ADP	=	Adenosine diphosphate
cAMP	=	3'-5'-cyclic adenosine monophosphate
PKA	=	Protein kinase A
PTK	=	Protein tyrosine kinase
DNA	=	Deoxyribonucleic acid
IRS	=	Insulin receptor substrate protein
PI3K	=	Phosphatidylinositol 3-kinase
PKB/AKT	=	Protein kinase B
MAPK	=	Ras–mitogen-activated protein kinase
mRNA	=	Messenger ribonucleic acid
p38MAPK	=	p38–mitogen-activated protein kinase
PKC	=	Protein kinase C
MEK	=	Mitogen-activated protein kinase kinase
PIP3	=	Phosphatidylinositol (3,4,5)-trisphosphate
G-6-Pase	=	Glucose-6-phosphatase
GSK-3	=	Glycogen synthase kinase 3
PP1	=	Protein phosphatase 1
p70s6k	=	p70-ribosomal S6 kinase
Cbl	=	Adaptor protein
SHC	=	Adaptor protein
PDK	=	Phosphoinositide-dependent kinase

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Received: 15 January, 2008 **Revised: 14 April, 2008** **Accepted: 16 April, 2008**