Review Article

Daidzein, its effects on impaired glucose and lipid metabolism and vascular inflammation associated with type 2 diabetes

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Abstract

Over the last decades, the incidence of type 2 diabetes (T2D) is increasing substantially. Emerging evidences from epidemiological studies have shown the association between higher intake of soy isoflavones and reduced risk of T2D and its associated health risks. Daidzein, a soy isoflavone, has been found to have a promising therapeutic potential in managing T2D pathophysiology. Fermented soybean is the major source of daidzein; however, it can also be formed via the consumption of its glycosylated moiety, daidzin with subsequent hydrolysis by intestinal bacterial enzyme. Many studies reported the prophylactic effect of daidzein on the improvement of hyperglycemia, insulin resistance, dyslipidemia, obesity, inflammation, and other complications associated with T2D. The molecular mechanisms underlying the action of daidzein include diverged pathways where daidzein has been shown to interact with several signaling molecules and receptors to achieve desirable effect. Although the specific molecular mechanism is still elusive, further studies are thus needed to understand it in detail. In this review, we discuss the antidiabetic potential of daidzein with respect to the evidences from various clinical, preclinical, and cell culture studies and the underlying molecular mechanism in a precise way to have a comprehensive account on this isoflavone with promising therapeutic potential. © 2018 BioFactors, 00(00):1–11, 2018

Keywords: type 2 diabetes; impaired glucose and lipid metabolism; vascular inflammation; daidzein; adjuvant therapeutic

Abbreviations: AMPK, 5'-adenosine monophosphate-activated protein kinase; AP1, activator protein 1; CCL2, chemokine (C-C motif) ligand 2; CPT1, carnitine-palmitoyltransferase1; CXCL2, chemokine (C-X-C motif) ligand 2; DHD, dihydoraidzein; FAS, fatty acid synthase; GLUT4, glucose transporter 4; G6Pase, glucose 6-phosphatase; G6PD, glucose-6-phosphate dehydrogenase; GK, glucokinase; GS, glycogen synthase; HOMA, homeostatic model assessment; IL1, interleukin-1; IL6, interleukin-6; IRS-1, insulin receptor substrate 1; IRS-2, insulin receptor substrate 2; JNK, c-Jun N-terminal kinase; MCP-1, monocyte chemoattractant protein-1; NOD, nonobese diabetic; NF-κB, nuclear factor kappa-B; O-DMA, desmethylangolensin; PARP1, poly [ADP-ribose] polymerase 1; PEPCK, phosphoenolpyruvate carboxykinase; PM, plasma membrane; PPARα, peroxisome proliferator-activated receptor alpha; PPARγ, peroxisome proliferator-activated receptor gamma; SCD, stearoyl-CoA desaturase; SREBP, sterol regulatory element-binding protein; T2D, type 2 diabetes; TNF-α, tumor necrosis factor alpha.

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1. Introduction

Type 2 diabetes (T2D) is a complex heterogeneous metabolic disorder affecting many people worldwide. The prevalence of this disorder is gradually increasing and posing threat to public health like an epidemic. T2D is characterized by both insulin deficiency and peripheral insulin resistance. Patients suffering from T2D are susceptible to several risk factors, like cardiovascular disease, coronary artery disease, renal failure, fatty liver, obesity, and neurological disorders. A number of therapeutic approaches are applied or suggested to manage T2D; but to what extent they are really able to manage all the complications is still a matter of debate. Even some of the conventional therapeutic approaches just have become useless.

Several epidemiological evidences reported that the incidence of T2D is lower in Asian populations compared to those seen in western countries and this might be due to the higher intake of fermented food products which are unique to traditional Asian diet. Many studies reported that people from several Asian countries like China, Japan, Thailand, and North Korea consume a very high amount of soy products (enriched in soy peptides and isoflavonones) and the mean intake of soy isoflavone was ranging between 6 and 75 mg/day while among Spain and Dutch populations it was approximately 0.4 mg/day and in United States the average intake was 0.3 mg/day [1–4]. These findings provide promising approaches to study and explore the beneficial role underlying the mechanism of actions of these bioactive soy isoflavononic compounds and related agents in attenuating or preventing T2D. Meanwhile, various studies have been conducted concerning the beneficial role of dietary soy isoflavones and their metabolites in lowering the high blood glucose level and other associated complications. It has been reported that genistin, daidzin, and glycitin are the three main soy isoflavones and among them the first two are the major ones available as sugars conjugated form (glycosides) in most soy foods in Asian cuisines [5,6]. Although found in a number of plants, soybeans and soy products are known as the principal source for isoflavones compounds. Up until recently, daidzein has been considered as one of the most important and highly studied isoflavones [7]. However, more recently attention has also been given to its metabolites, like desmethyleangolensin (DMA), dihydrodaidzein (DHD), and cis-4-OH-equol, which are formed by intestinal bacteria [8]. Among the daidzein metabolites, equol has been known to be very effective in the regulation of insulin resistance and functional similarities with endogenous estrogen. In plants, daidzein exists in conjugation mainly with glucose, but other than glucose they may also conjugate with 6′-O-malonyl or 6″-O-acetylglucose [13,14]. Daidzein can be metabolized to form several derivatives, including 0-desmethyleangolensin (0-DMA,2′, 4′,4″-trihydroxy-α-methyl deoxynbenzoin), DHD (4′,7-dihydroxyisoflavanone), and cis-4-OH-equol (4′7-dihydroxyisoflavan-4-ol) [15]. These are formed mainly by the process of demethylation and reduction by intestinal bacteria.

2. Biochemistry

Daidzein (7-hydroxy-3-(4-hydroxyphenyl)-4Hchromen-4-one) is produced in plants through the phenyl propanoid pathway and exerts defense responses to several pathogenic attacks in plants [11]. It belongs to a class of compounds known as isoflavonoid containing benzene ring connected to a chromone moiety [12]. It is also classified as phytoestrogen because of its structural and functional similarities with endogenous estrogen. In plants, daidzein exists in conjugation mainly with glucose, but other than glucose they may also conjugate with 6″-O-malonyl or 6′-O-acetylglucose [13,14]. Daidzein can be metabolized to form several derivatives, including 0-desmethyleangolensin (0-DMA,2′, 4′,4″-trihydroxy-α-methyl deoxynbenzoin), DHD (4′,7-dihydroxyisoflavanone), and cis-4-OH-equol (4′7-dihydroxyisoflavan-4-ol) [15]. These are formed mainly by the process of demethylation and reduction by intestinal bacteria.

3. Food sources of daidzein

Daidzin is present predominantly in the form of glucosides in various plants, including red clover (Trifolium pratense), alfalfa (Medicago sativa), soybean (Glycine max), and some legumes (Leguminosae) [12]. Among them soybean and soybean products are considered as the most abundant source of daidzein [16,17]. Soybean contains approximately 0.1–5 mg of total isoflavones per gram [18]; however the amount varies depending upon types of soybean, area wise distribution, and harvesting year [16,19,20]. In fermented soybean, the amount of daidzin is higher than those seen in nonfermented soybean because fermentation increases the level of active aglycone form [7]. Various microorganisms including Bacillus sp., Rhizopus sp., and Aspergillus sp. have been reported to involve in the aglycosylation of isoflavone during fermentation process [21–23]. Besides soy products, daidzein can also be found in a large amount in many nutritional supplements, body building beverages, sports drinks, different infant formulas, etc. [24]. The presence of daidzein (both glycoside and aglycone forms) has also been reported in kudzu root (Pueraria radix), a popular traditional Chinese medicine commonly used as dietary supplements [25–27].

4. Absorption, bioavailability, and metabolism of daidzein

In plants, daidzein exists in the form of glycosides (known as daidzin) as biologically inactive component and remain unmodified during various food preparations [28]. Gut microflora plays an important role in the bioconversion of daidzin. After consumption, the glycosidic linkage of daidzin is hydrolyzed in the intestine by lactase enzyme of small intestine or by β-glucosidases of intestinal bacteria, especially Bifobacterium and Lactobacillus strain, followed by the formation of bioactive aglycone moiety, daidzin [29–31]. After aglycosylation, daidzein is further converted to its derivatives, namely DHD, DMA, and cis-4-OH-equol through the
process of demethylation and reduction by various bacterial strains found in the human gut [15]. Daidzein has been reported to be converted into DHD through the process of hydrogenation by Coprobacillus sp. MRG1, Clostridium sp. TM-40 [32,33]. DHD can further be metabolized to form DMA and equol through the reductive cleavage of heterocyclic ring and two step deoxygenation reaction, respectively [34]. Several studies have reported that Eubacterium ramulus, Clostridium sp. HGH136, and Clostridium sp. SY8519 are efficient O-DMA producers [35–37]. On the contrary, bacterial genera including Slakedia sp. and Eggertellha sp. belonging to Coriobacteriaceae family have been found to be involved in the production of equol from DHD [38,39]. Anaerobic bacterium (Mt1B8) from the mouse intestine has been reported to convert approximately 80% daidzein to equol during 24 h incubation [40]. The aglycone moiety is absorbed from the gastrointestinal tract by passive diffusion in the upper part of the small intestine.

Kwon et al. [7] reported that fermented soy foods may enhance the absorption of isoflavones among the people who consume fermented soybean compared to those consuming non-fermented soybean, which may be due to the probiotic effects of fermented foods which may result in an increase in the gut bacterial population.

In the circulation, daidzein can bind nonspecifically to human serum proteins. Prasain et al. [41] observed a very low concentration of daidzein in plasma as free unbound form (~12%) and it has been recommended that only unbound fraction may have potential to exert target specific biological responses since they are free to bind with their target receptor. Daidzein has also been reported to be distributed among several tissues, like kidney, liver, muscle, placenta, and mammary gland [41]. Daidzein can cross the blood-brain barrier and a detectable concentration was reported in brain within first hour of its administration [42]. LC MS/MS analyses showed that daidzein can reach in various areas of brain, including the hippocampus, striatum, cortex, cerebellum, brain stem and hypothalamus [43]. In liver, daidzein is generally converted to glucuronosides, which are either re-excreted through the bile or reabsorbed by enterohepatic recycling or excreted unmodified in the urine [16]. The rate of absorption and metabolism may differ greatly among various human populations and even among the individual of a particular population having different diet. Among children the absorption is more efficient compared to those seen in adults [44,45]. Thus several factors, like age, food habit, and bacterial population in gut may influence the daidzein absorption, bioavailability, and metabolism. Figure 1 represents the schematic diagram about the formation and metabolism of daidzein.

5. Effect of daidzein on T2D pathophysiology

5.1. In vitro studies
Various in vitro studies examined the efficacy of daidzein as an antidiabetic or antihyperglycemic agent against the T2D pathophysiology. Using L6 myotube cell culture model, Cheong et al. [46] demonstrated a dose-dependent effect of daidzein (25-100 μM) on intracellular glucose uptake in absence of insulin. Cho et al. [9] further showed that treatment with daidzein at a dose of ≥10 μM significantly enhanced the adipocyte differentiation and insulin induced glucose uptake in 3T3L1 adipocyte cells. Interestingly, daidzein treatment was effective at a dose of 1–10 μM in mesenchymal stem cells 10T1/2 suggesting that 3T3L1 cells requires a higher doses to exert equipotent in vivo effects compared to mesenchymal 10 T1/2 stem cell line. Beside this, daidzein metabolite equol also increased the adipocyte differentiation and glucose uptake in both 3T3L1 adipocyte and 10 T1/2 stem cells [9]. Park et al. [47] reported a significant inhibitory effect of daidzein against α-glucosidase and α-amylase activities and the IC50 values of daidzein against α-glucosidase and α-amylase were found to be 0.048 and 0.301 mmol, respectively. An increase in vascular inflammation plays a critical role in developing insulin resistance. Sakamoto et al. [48] examined the effect of daidzein on the markers of pro-inflammatory cytokines in the co-cultures of 3T3L1 adipocyte and RAW264 macrophage. Results showed that that daidzein (25 μM) treatment significantly inhibited the mRNA expression of pro-inflammatory cytokines, CCL2 and IL6 in adipocytes induced by co-culture. Daidzein treatment also decreased the CCL2 and IL6 mRNA expression in RAW264 macrophages stimulated with either palmitate (400 μM) or conditioned medium from 3T3L1 adipocytes. The anti-inflammatory effect of daidzein has also been examined by using TNFα-treated (20 ng/mL) murine MLE-12 epithelial cells. It was observed that daidzein treatment (10 μM) blocked the transcriptional activation of pro-inflammatory genes and decreased the mRNA level of Cxcl2 in TNFα-treated cells [49]. Table 1 represents the cell culture studies about the effect of daidzein or equol supplementation on glucose and lipid metabolism and vascular inflammation associated with T2D pathophysiology.

5.2. Preclinical studies
Several in vivo studies examined the beneficial effect of daidzein intake in the regulation of glycemic status among different diabetic animal models. Choi et al. investigated the therapeutic effect of daidzein in the regulation of glucose homeostasis in nonobese diabetic (NOD) mice. Results showed that dietary supplementation of daidzein at a dose of 0.2 g/kg diet for 9 week caused a marked reduction in fasting blood glucose with concomitant decrease in plasma insulin and C peptide levels compared to diabetic control. In daidzein-treated individuals, a significant increase in hepatic gluokinase (GK) and a decrease in G6Pase and PEPCCK activities were also observed suggesting a beneficial effect of daidzein supplementation in reducing both glycoenzolysis and neoglucogenesis in diabetic mice [50]. Using db/db mice another study by Park et al. also demonstrated that daidzein (0.2 g/kg diet, 6 weeks) supplementation improved the glucose and lipid metabolism and regulated the hepatic glucose (GK, G6Pase, and PEPCCK) and lipid (FAS, CPT, β-oxidation) regulating enzyme activities compared to those seen in diabetic
control group [51]. Cheong et al. [46] further demonstrated that 0.1% daidzein supplementation in the diet for 4 weeks also suppressed the rise in fasting glucose and lipid levels and insulin resistance compared to the diabetic (db/db mice) control group. In the same study, Cheong et al. further showed that treatment with daidzein also caused a reduction in nonfasting blood glucose and urinary glucose excretion in KK-AY/Ta jcl mice, a potent animal model of T2Ds [46]. In ovariectomized rats,

**TABLE 1**

*Effect of daidzein on T2D pathophysiology in cell culture studies*

<table>
<thead>
<tr>
<th>Model of study</th>
<th>Dose of daidzein/equol</th>
<th>Outcome</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>L6 myotubes</td>
<td>0-100 μM for 4 hour against high glucose exposure</td>
<td>Increase in AMPK phosphorylation followed by GLUT 4 translocation and glucose uptake</td>
<td>[46]</td>
</tr>
<tr>
<td>3 T3 L1 adipocytes</td>
<td>0–100 μM for 5 days against high glucose exposure</td>
<td>Dose-dependent increase in adipocyte differentiation and insulin stimulated glucose uptake</td>
<td>[9]</td>
</tr>
<tr>
<td>10 T1/2 cells</td>
<td>0–20 μM for 8 days</td>
<td>Increase in adipocyte differentiation and accumulation of intracellular triacylglycerols</td>
<td>[9]</td>
</tr>
<tr>
<td>Co-culture of 3 T3 L1 adipocytes and RAW 264 macrophages</td>
<td>25 μM for 24 hours against palmitate treatment</td>
<td>Attenuation of the expression of pro-inflammatory genes, namely Ccl2 and Il6 via PPARα/γ and JNK pathways</td>
<td>[48]</td>
</tr>
<tr>
<td>3 T3 L1 adipocytes</td>
<td>12.5 μM– 50 μM for 6–8 days</td>
<td>Up-regulation of PPARγ activity followed by the downregulation of pro-inflammatory adipocytokines, MCP1 and TNFα</td>
<td>[75]</td>
</tr>
<tr>
<td>Murine lung epithelial cells</td>
<td>10 μM for 6 h against TNFα stimulation</td>
<td>Downregulation of PARP activity followed by suppression of pro-inflammatory chemokine, Cxcl2</td>
<td>[49]</td>
</tr>
</tbody>
</table>
Daidzein intake at a dose of 50 mg/kg/day decreased the gain in body weight, visceral fat, and the levels of serum insulin, glucose, lipid, insulin resistance, and the secretion of cytokines, namely TNF-α, leptin, and adiponectin [52]. Another study with high fat diet fed C57BL/6J mice reported the synergistic effect of daidzein and glycitin (0.06% mixture in diet, in the ratio of 3:1) in controlling diabetes and obesity-related complications. Results showed a significant reduction in body weight gain, fat content in adipose tissue, and the levels of fasting glucose, HbA1c, insulin, and total cholesterol [53]. In a study of C57BL/6j lean mice using radio labeled (C14) glucose, Meezan et al. demonstrated that daidzein supplementation (75 mg/kg body weight) significantly increased glucose uptake in various tissues. Moreover, daidzein supplementation also increased the glycogen synthesis in various tissues, like liver, heart, and red blood cells as evidenced by the incorporation of C14 into glycogen [54]. Some studies further elucidated the antidiabetic effect of equol, a daidzein derivative [10]. Supplementation with equol (0.05% in diet) suppressed the rises in fasting blood glucose, cholesterol, triglyceride, lipid peroxidation, and hepatic triglyceride levels and improved the impaired glucose tolerance in ob/ob mice. Thus, findings from these preclinical studies support strong evidences in favor of daidzein as an adjuvant therapeutic for the regulation of glucose homeostasis in T2D. However, detailed in vivo experimental studies are still required to explore the action of daidzein in different aspects. Table 2 represents the preclinical studies about the outcome of daidzein or equol in T2D pathophysiology.

### 5.3. Clinical studies

A number of epidemiological studies and clinical trials have been conducted among different populations to investigate the effects of soyabean isoflavones on the management of the risk of T2D. Results demonstrate a significant beneficial effect of soybean isoflavone on regulating the glycemic parameters in diabetic patients [55,56]. Beside this, improvement in lipid profile, kidney function, endothelial function, and blood pressures has also been observed among the population supplemented with soybean isoflavones [57–61]. A case–control study among Vietnamese adults (aged 40–45 years) showed that dietary

### TABLE 2

**Effect of daidzein on T2D pathophysiology in preclinical studies**

<table>
<thead>
<tr>
<th>Model of study</th>
<th>Dose of daidzein/equol</th>
<th>Outcome</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female nonobese diabetic mice, 9 weeks old</td>
<td>0.2 g/kg body weight for 9 weeks</td>
<td>Reduction in plasma glucose level, insulin and C-peptide and higher hepatic GK activity and lower G6Pase and PEPCK activities</td>
<td>[50]</td>
</tr>
<tr>
<td>Male C57BL/KsJ-lepr&lt;sup&gt;db/db&lt;/sup&gt;/lepr&lt;sup&gt;db&lt;/sup&gt; mice, 6 weeks old</td>
<td>0.2 g/kg body weight for 6 weeks</td>
<td>Improvement of the glucose and lipid metabolism and regulation of the hepatic glucose (GK, G6Pase, and PEPCK) and lipid (FAS, CPT, β-oxidation) regulating enzyme activities</td>
<td>[51]</td>
</tr>
<tr>
<td>Male db/db mice, 6 weeks old</td>
<td>0.1% in the diet for 4–5 weeks</td>
<td>Suppression of the rise in fasting blood glucose</td>
<td>[46]</td>
</tr>
<tr>
<td>Male KK-Ay/Ta Jcl mice, 4 weeks old</td>
<td>0.1% in the diet for 4–5 weeks</td>
<td>Reduction of nonfasting blood glucose level and urinary glucose excretion.</td>
<td>[46]</td>
</tr>
<tr>
<td>C57BL/6j lean mice, 4–5 weeks old</td>
<td>75 mg/kg body weight</td>
<td>Elevation of C14 glucose uptake and glycogen synthesis</td>
<td>[54]</td>
</tr>
<tr>
<td>Ovariectomized rats, 8 weeks old</td>
<td>50 mg/kg body weight/day for 12 weeks</td>
<td>Improvement of plasma lipid profile, insulin resistance and reduction of inflammatory cytokine secretion</td>
<td>[52]</td>
</tr>
<tr>
<td>C57BL/6 J mice, 6 weeks old</td>
<td>0.06% mixture (daidzein: glycitin = 3:2) in diet for 92 days</td>
<td>Reduction of body weight gain, fat content in adipose tissue, total cholesterol, fasting glucose, HbA1c, and insulin levels</td>
<td>[53]</td>
</tr>
<tr>
<td>Male C57BL/6 J-ob/ob mice, 6 weeks old</td>
<td>0.05% Equol in the diet for 3 weeks</td>
<td>Suppression of the rise in fasting blood glucose, plasma cholesterol, triglyceride levels, and improvement of impaired glucose metabolism</td>
<td>[10]</td>
</tr>
</tbody>
</table>

Das et al.
intake of daidzein was significantly lower among diabetic population compared to controls and it was also associated with reduced risk of T2D [62]. Similarly, Ho et al. [63] reported that supplementation with soy isoflavone containing 46.4% daidzein have a favorable effect on lowering fasting glucose in postmenopausal Chinese women. The effect of daidzein metabolite, equol was also evaluated on the metabolic profiles among Japanese overweight/obese men and women. Results showed that equol intake at a dose of 10 mg/day for 12 weeks caused a significant improvement in HbA1c among the treated individuals compared to placebo [55]. In a survey-based study among United States pregnant women, it was found that urinary concentration of total isoflavone including daidzein, genistein, DMA, and equol were inversely associated with fasting glucose, insulin and homeostatic model assessment (HOMA) of insulin resistance (HOMA-IR) [56]. A larger prospective cohort study conducted among Chinese women reported that soybean and soymilk intake was inversely associated with the prevalence of T2D [64]. However, the beneficial effects of soy isoflavones on glucose metabolism were not found in some short-term trials and some of the studies were statistically insignificant [65,66]. Therefore, detail long-term clinical trials are required to explore the effect of daidzein on glucose metabolism among human population. Table 3 summarizes the clinical studies on the effect of daidzein or equol in T2D pathophysiology.

### 6. Possible molecular mechanism

The molecular mechanism underlying the action of daidzein in minimizing diabetes and its related complications remains enigmatic till date. However, several experimental evidences reported its mechanism of action in various aspects including glucose and lipid metabolism and vascular inflammation.

#### 6.1. Effect of daidzein on glucose metabolism

Various in vivo and in vitro studies have been performed till date to explore the role of daidzein in the regulation of glucose metabolism. Several studies have confirmed that daidzein promotes glucose uptake in adipocytes and muscle cells and suppresses the rise of serum glucose level [9,10,46,50,51]. Cheong et al. [46] reported the beneficial effect of daidzein in improving glucose homeostasis in high glucose treated L6 myotube cells. Results showed that treatment with the optimum dose of daidzein caused a significant elevation in the ratio of glucose transporter 4 (GLUT4) to Na+/K+ATPase in the plasma membrane (PM) fraction of L6 myotubes suggesting the potential role of daidzein in promoting glucose uptake via GLUT4 translocation from intracellular microvesicles to PM. Moreover, the authors also investigated the role of adenosine monophosphate activated protein kinase (AMPK), the key regulator of cellular energy homeostasis in mediating the beneficial action of daidzein on glucose uptake. It was found that daidzein supplementation led to a significant phosphorylation of AMPK in high glucose treated myotubes, which in turn promoted GLUT4 translocation and subsequent glucose uptake. In another study, Cheong et al. [10] reported the promising role of equol, a daidzein derivative, in serum glucose uptake via GLUT4 translocation, and AMPK activation. During in vitro studies, equol was found to regulate the expression levels of major genes involved in gluconeogenesis and glycogenesis pathways. Equol treatment significantly decreased the expression levels of phosphoenolpyruvate carboxykinase (PEPCK) and glucose 6-phosphatase (G6Pase) and increased the glycogen synthase (GS) and

#### TABLE 3

<table>
<thead>
<tr>
<th>Model of study</th>
<th>Dose of daidzein/Equol</th>
<th>Outcome</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vietnamese adults, aged 40–45 years</td>
<td>5.2–9.8 mg/day</td>
<td>Reduced risk of T2D.</td>
<td>[62]</td>
</tr>
<tr>
<td>Postmenopausal Chinese women, aged 48–62 years</td>
<td>Mid-dose 40 mg/day; High dose 80 mg/day (daidzein 46.7%) for 1 year</td>
<td>A favorable effect on lowering fasting glucose by higher doses of daidzein supplementation</td>
<td>[63]</td>
</tr>
<tr>
<td>Placebo n = 68, Mid-dose n = 68, High-dose n = 67</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Japanese overweight/obese men and women</td>
<td>10 mg/ day (Equol) for 12 weeks</td>
<td>Improvement in HbA1c compared to placebo</td>
<td>[55]</td>
</tr>
<tr>
<td>Pregnant U.S. women, average age 28y, n = 299</td>
<td></td>
<td>Inverse association between urinary concentration of total isoflavone including daidzein and fasting glucose, insulin, and HOMA-IR</td>
<td>[56]</td>
</tr>
<tr>
<td>Chinese women, aged 30–70, placebo n = 54 daidzein n = 55</td>
<td>50 mg/ day for 24 weeks</td>
<td>No significant differences in fasting glucose, HbA1c, insulin, HOMA-IR</td>
<td>[64]</td>
</tr>
</tbody>
</table>
enhanced basal glucose uptake in high glucose diet-fed mice. Cho et al. [9] demonstrated that daidzein and its derivative equol significantly increased the adipocyte differentiation via activation of PPARγ, the key regulator of the differentiation event. The authors also addressed that the increased transcriptional activity of PPARγ*per se* led to a higher expression of downstream signaling molecules, such as GLUT4 and insulin receptor substrate (IRS-1) followed by enhanced basal glucose uptake and insulin sensitivity. Some studies have performed enzyme specific biochemical assays to access the beneficial effect of daidzein in regulation of glucose metabolism [50,51]. It was observed that upon daidzein supplementation, hepatic GK activity was significantly elevated while G6Pase and PEPCK activity was markedly reduced leading to the inhibition of gluconeogenesis and upregulation of glycolysis in NOD and db/db mice [50,51]. Although much remains to be elucidated regarding the molecular mechanisms underlying the beneficial role of daidzein in glucose metabolism, these studies altogether improves our understanding about the critical function played by daidzein in reducing the risk of diabetes. Figure 2 demonstrates the probable molecular mechanism underlying the beneficial role of daidzein on glucose metabolism.

6.2. Effect of daidzein on lipid metabolism
The alteration of lipid metabolism in T2Ds has been found to play an important role in the development of hepatic insulin resistance [67–69]. A series of studies in the literature have explored the potential role of daidzein and its molecular mechanism of action in regulating lipid metabolic pathways during diabetic condition (Fig. 3). Park et al. [51] investigated the role of daidzein in hepatic lipid metabolism by measuring the activities of lipid regulating enzymes. The authors observed that supplementation with daidzein significantly reduced the activities of hepatic lipid regulating enzymes, such as fatty acid synthase (FAS) and carnitine palmitoyltransferase (CPT) in type 2 diabetic db/db mice. Choi et al. [50] reported the important role of daidzein in maintaining glucose homeostasis in type 1 diabetic NOD mice by significantly elevating the activities of lipogenic malic enzyme and glucose-6-phosphate dehydrogenase (G6PD) and lowering the hepatic CPT level. Takahashi et al. [70] showed that soy isoflavones (including daidzein) have the potential to modulate the expression of SREBP-1 isoforms and the downstream signaling molecules involved in lipogenesis. Results showed that supplementation with soy isoflavones (daidzein; 59.9% in weight) in an optimum dose caused a significant decrease in the gene expression levels of FAS, stearoyl-CoA desaturase (SCD) 1, and 6 leading to a decrease in lipogenesis. Daidzein has also been found to promote increased expression of PPARγ which is known to play a major role in modulating the expression of several key molecules involved in lipid and glucose metabolism during the onset of T2D [9]. Cheong et al. reported that treatment with daidzein metabolite, equol significantly suppressed the abnormal elevation in the expression of FAS and SCD and increased the expression of CPT I followed by an improved glucose tolerance in the obese diabetic ob/ob mice.

6.3. Effect of daidzein on inflammation
Sub-clinical, chronic, low-grade inflammation plays an important role in the pathogenesis of insulin resistance, T2Ds, and cardiovascular diseases [71,72]. Many studies have reported the enhanced expression of several pro-inflammatory cytokines including TNF-α, IL-1, IL-6, and monocyte chemoattractant protein-1 (MCP-1) in the context of insulin resistance, obesity, T2Ds, and cardiovascular diseases [71,73–75]. Daidzein treatment has been shown to suppress the activation of several inflammatory mediators associated with above-mentioned
diseases [48,49,52,75]. Using adipocyte cell culture model, Sakamoto et al. [75] reported that treatment with daidzein upregulated the adipogenic differentiation and gene expression of PPARγ and adiponectin and downregulated the MCP-1 gene expression and its secretion. Additionally, the authors reported that in high fat diet-fed mice daidzein administration also upregulated the gene expression of PPARγ and adiponectin and downregulated the MCP-1 and TNF-α gene expression in fat tissue and thereby inhibited hypertrophy in fat cell size and improved insulin sensitivity [75]. In another study, Sakamoto et al. [48] further reported the role of PPAR and JNK pathways in mediating the anti-inflammatory effect of daidzein. Results showed that daidzein treatment caused a significant increase in PPARα transcriptional activity and a decrease in JNK phosphorylation followed by down-regulation in the mRNA expression of Ccl2 and IL6 in palmitate-treated macrophage cells. Moreover, daidzein supplementation in adipocyte and macrophage co-culture system downregulated the gene expression of pro-inflammatory cytokines and upregulated adiponectin gene expression by increasing PPARγ transcriptional activity. Anti-inflammatory activity of daidzein has also been examined by using TNFα-treated murine MLE-12 epithelial cells [49]. Results showed that daidzein treatment significantly inhibited the TNFα-induced increase in Cxcl2 expression and activity and nuclear factor kappa-B (NF-κB) transcriptional activity and prevented the TNF-α-induced protein PARylation. Using PARP1 expression plasmid and NF-κB-luc reporter plasmid, results suggested that the anti-inflammatory action of daidzein in murine lung epithelial cells was mediated via a direct interaction with PARP-1, which inhibits PARylation of NF-κB and thus prevented the transcriptional modulation of pro-inflammatory chemokines such as Cxcl2. Combining all evidences, it can be suggested that daidzein treatment could ameliorate the inflammatory condition by modulating the expression of several inflammatory markers via activating PPARγ and inhibiting JNK, PARP, NF-κB signaling pathways (Fig. 4).

7. Conclusion

Combining the available evidences till date, this review will help us in understanding the beneficial role of daidzein for the improvement of diabetic condition and its related complications. More exactly daidzein supplementation has been shown to have profound effect on the amelioration of insulin resistance, obesity associated complications, inflammation, alteration of plasma lipid profile, dyslipidemia, etc. Epidemiological observations, preclinical, clinical, and cell culture studies support the validity of fact that daidzein could be a promising bioactive principle in the management of T2D and its associated complications (Fig. 5). Food sources, bioavailability, absorption, and metabolism of daidzein have also been discussed in this review. Daidzein has been found to be functional only in its aglycone form and it would not be
active unless the glycosidic bond of daidzin is being cleaved. Therefore, fermented soybean has been considered a very effective source of bioactive daidzein and other isoflavones since fermentation causes breakdown of glycosidic linkage of those glycosides. Although several clinical studies addressed the association between consumption of soy product and lower risk of T2D; however, some limitations still exist regarding the bioavailability and dose standardization. Moreover, short-term clinical trials are mostly observational and not significant. In this respect, more long-term interventional studies are required to confirm the effectiveness of daidzein in regulating the glycemic status among T2D patients. In addition, more studies are required in elucidating the detail molecular mechanism underlying the role of daidzein in regulating glycemic status, lipid profile, and inflammation. In contrast to daidzein, another soy isoflavone, genistein has also been considered as effective therapeutic agent in preventing T2D and the additive effects of these two isoflavones have also been reported in the literature, but some studies have reported the adverse effects of genistein including carcinogenesis. Synergistic reported in the literature, but some studies have reported the additive effects of these two isoflavones, including carcinogenesis. Synergistic study of daidzein with other group of bioactive compounds may also be helpful for minimizing diabetic complications and that could be a promising area to shed more light in research with diabetes and its associated complication. Further studies are thus needed to ascertain its efficacy profile or therapeutic potential in different aspects to control the disease worldwide.

Conflict of Interest
The authors have declared that no conflict of interest exists.

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