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Effect of Genistein and Daidzein on Glucose Uptake in Isolated Rat Adipocytes; Comparison with Respective Glycones

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Abstract

Soy and soy foods are a rich source of isoflavones, which possess several biological activities. The effect of soy isoflavones, genistin and diadzin and their respective aglycones, on glucose uptake in adipocytes isolated from normal or high-fat fed rats was examined. As expected, insulin stimulated glucose uptake in a concentration-dependent manner. However, genistin and daidzin and their aglycones inhibited glucose uptake in a concentration-dependent (25~100 μM) manner. In a time-course response, the aglycones significantly inhibited glucose uptake throughout 3 hr (after 30, 60, 120, 180 min), whereas the glycones only significantly inhibited the glucose uptake after 120 min and 180 min in the isolated rat adipocytes. Thus, the glucosides of genistein and daidzein, i.e. genistin and daidzin, were much less effective in inhibiting glucose uptake than their aglycones. In addition, genistin and daidzin did not significantly affect the insulin-stimulated glucose uptake, whereas genistein and daidzein did significantly inhibited glucose uptake compared to the vehicle control group by 47.5% and 24.8%, respectively ($p < 0.05$). The isoflavones also significantly inhibited glucose uptake in adipocytes isolated from rats fed a high-fat diet (50% of total calorie intake) when compared to the vehicle control. Finally, the isoflavones were found to enhance lipolysis in adipocytes isolated from high-fat fed rats, where the glycerol released by the aglycones was also higher than that released by the glycones. The current results showed that the inhibitory effect of daidzein on glucose uptake was very similar to that of genistein. The aglycones were more potent in inhibiting the uptake of glucose and a more potent stimulator of lypolysis than the glycones in adipocytes isolated from high-fat fed rats.

Key words: soy isoflavones, glucose uptake, adipocytes, lipolysis

INTRODUCTION

There is growing interest in the potential health effects of soy and soy isoflavones, as epidemiological studies have associated a diet rich in isoflavones with a lower risk of certain diseases, including breast and prostate cancer, osteoporosis, and cardiovascular disease (1-5).

The major soy isoflavones are genistin and daidzin (β-glycosides), and their aglycone forms, genistein and daidzein (Fig. 1). After ingestion, the β-glycosides undergo further metabolism by intestinal microflora (6,7), and through the action of bacterial glycosidases, and they are converted to aglycones (8-10).

Recent studies have also provided evidence that soy consumption alleviates some of the symptoms associated with type 2 diabetes, such as insulin resistance and impaired glycemic control (11,12) Moreover, several other researchers have reported that genistein, a tyrosine kinase inhibitor (13) that was not found to inhibit insulin receptor kinase activity in isolated rat adipocytes, completely inhibited insulin-stimulated glucose oxidation (14). While, daidzein (another major soy isoflavone), which does not have a hydroxyl group at the 5-position of genistein, is commonly used as inactive analogue of genistein with respect to the inhibition of tyrosine kinase *in vitro* (15).

Obesity, although multifactorial, may be induced by the chronic ingestion of a high-fat diet and is a prevalent cause of insulin resistance and type 2 diabetes (16). In particular, if obesity is associated with abdominal adiposity, this is an important determinant of insulin resistance and represents the most important risk factor for type 2 diabetes and metabolic syndrome (17). In rats, a long-term high-fat diet leads to a severe insulin re-

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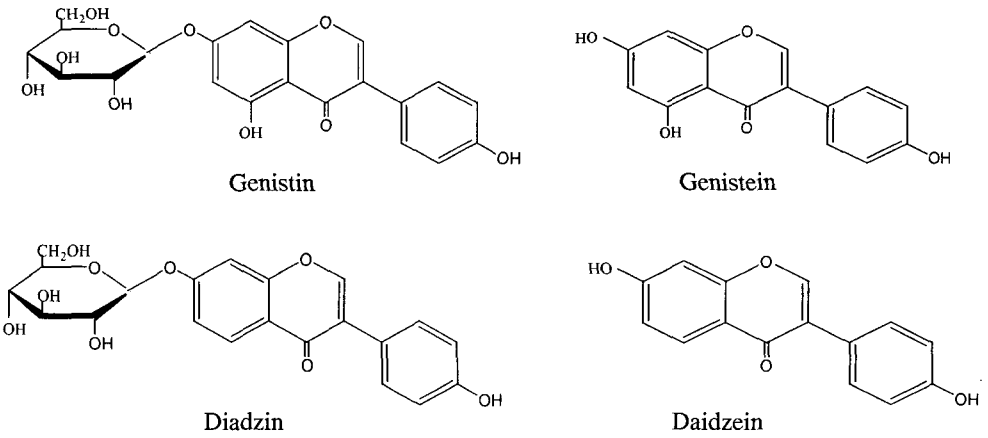

Fig. 1. Structures of soy isoflavones.

sistance, yet not diabetes (18). Accordingly, the purpose of this study was to elucidate the effect of genistein and daidzein on glucose uptake by comparing the effects of the glycones in adipocytes isolated from normal or high-fat fed rats.

MATERIALS AND METHODS

Reagents

The human insulin was purchased from Novo Co. (Copenhagen, Denmark), while the genistin, genistein, daidzin, bovine serum albumin (fraction V), collagenase, cytochalasin B, and silicon oil were all purchased from Sigma Co. (St. Louis, MO, USA). The daidzein was from Fluka Co. (Buchs, Switzerland), and the 2-deoxy [^{3}H]-glucose was purchased from New England Nuclear Corp. (Boston, MA, USA). All other chemicals were of reagent grade or better.

Animals

Sprague-Dawley male rats weighing between 180~200 g were purchased from Bio Genomics, Inc. (Seoul, Korea) and fed a standard laboratory chow diet *ad libitum*. To obtain adipocytes from high-fat fed rats, Sprague-Dawley male rats weighing between 50~60 g were separately purchased from Bio Genomics, Inc. (Seoul, Korea) and fed a high-fat diet (Table 1, 50% of total calorie intake) for 7~9 weeks. The animals were individually housed in stainless steel cages in a temperature (22±2°C) and light/dark (12 hr/12 hr) controlled room. The animals were then sacrificed and the adipocytes isolated.

Preparation of isolated adipocytes

The cell isolation was performed according to the methods of Rodbell (19) and Olefsky (20), and the experiments carried out in accordance with the ethical rules approved by Kyungpook National University. The

Table 1. Composition of high-fat diet (g/100 g diet)

Components	High-fat diet
Casein	20.0
D,L-Methionine	0.3
Corn starch	15.0
Sucrose	27.5
Cellulose powder	5.0
Corn oil	5.0
Lard	22.5
Mineral mixture[1)]	3.5
Vitamin mixture[2)]	1.0
Choline bitartrate	0.2

[1)]AIN-76 mineral mixture (Harlan Teklad Co., USA).
[2)]AIN-76 vitamin mixture (Harlan Teklad Co., USA).

rats were slaughtered by decapitation and the epididymal fat pads removed. The isolated fat cells were prepared by shaking at 37°C for 90 min in a Krebs-Ringer Hpeps (KRH) buffer (pH 7.4) containing 131.5 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.25 mM $MgSO_4$, 2.5 mM NaH_2PO_4, 10.0 mM Hepes, 1% bovine serum albumin (fraction V), 2 mM pyruvate, and 1 mg/mL collagenase (from Clostridium histolyticum, type II). After isolation, the cells were washed several times with the same buffer but without collagenase and filtered through a nylon mesh (200 μM pore size). The number of cells was then counted using a hematocytometer.

2-Deoxyglucose uptake assay

The isolated adipocytes (2×10^5 cells/mL) were pre-incubated in 1 mL of a KRH buffer at 37°C for 20 min, then incubated with DMSO (vehicle), genistin, daidzin, genistein, or daidzein. The glucose uptake reaction was initiated by adding 0.125 mM 2-deoxyglucose containing 2-deoxy[^{3}H]-glucose (2-DG). After incubation at 37°C for 3 min, the reaction was terminated by adding 40 μM Cytochalasin B (Sigma). The adipocytes were then separated from the medium by centrifugation (16,000×g, 2

min) through silicon oil, and the glucose uptake assessed based on the uptake of [^{3}H]-glucose into the adipocytes by counting in a liquid scintillation counter (Packard TriCarb 2500TR, Downer's Grove, IL).

Lipolysis in adipocytes

The adipocytes (10^6 cells/mL) were incubated at 37°C for 90 min in a KRH buffer, then incubated with DMSO, genistin, daidzin, genestein, and daidzein, respectively. Thereafter, the adipocytes were aspirated and the intensity of lipolysis ascertained as the amount of glycerol released from the cells into the incubation medium. The concentration of glycerol was determined using the Boehringer Mannheim test.

Statistical analysis

The data were expressed as the mean ± SEM, and the significance of the differences between the means, set at $p<0.05$, was assessed using a one-way analysis of variance and Duncan's multiple-range test or Student's *t*-test using the SPSS program.

RESULTS

Dose-dependent effects of soy isoflavones on 2-DG uptake in rat adipocytes

Insulin stimulated glucose uptake in a dose-dependent manner (0.001 ~ 1 μM) when compared to the basal level (Fig. 2). However, when the cells were treated with various concentrations (25 ~ 200 μM) of the four isoflavones, a marked inhibition of glucose uptake was observed at each concentration compared to the vehicle control (Fig. 3). Genistein was the most effective glucose uptake inhibitor at concentrations of 25, 50, and 100 μM, while genistin and daidzin were relatively less effective at concentrations from 25 to 200 μM. Nonetheless, daidzein was as effective as genistein in inhibiting glucose uptake in the adipocytes when its concentration was increased to 100 μM and 200 μM. Therefore, overall, genistin and daidzin, and their aglycones significantly inhibited glucose uptake in a concentration-dependent manner at ≤100 μM.

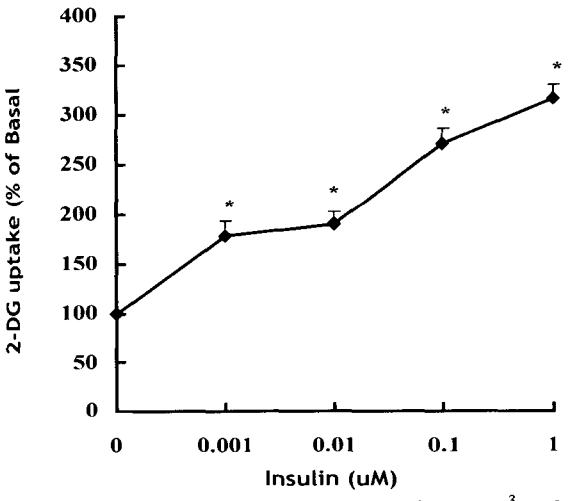

Fig. 2. Effect of different doses of insulin on [^{3}H]-2-DG uptake in isolated rat adipocytes. The cells were incubated with various insulin concentrations in a KRH buffer at 37°C for 30 min, then further incubated for 3 min in the same buffer containing [^{3}H]-2-DG. The radioactivity incorporated into the cells was measured. Each point represents the mean ± SEM for five repetitions. *$p<0.05$ vs. basal time by Student's *t*-test.

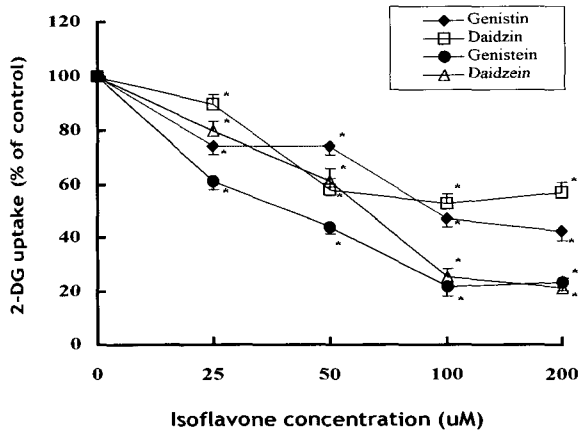

Fig. 3. Dose effects of isoflavones on [^{3}H]-2-DG uptake in isolated rat adipocytes. The cells were incubated with various isoflavone concentrations in a KRH buffer at 37°C for 3 hr, then further incubated for 3 min in the same buffer containing [^{3}H]-2-DG. The radioactivity incorporated into the cells was measured. Each point represents the mean ± SEM for five repetitions. *$p<0.05$ vs. control by Student's *t*-test.

Time-course effect of soy isoflavones on 2-DG uptake in rat adipocytes

Genistin and daidzin only inhibited glucose uptake significantly after 120 min and 180 min when compared to the control, whereas genistein and daidzein exhibited an inhibitory effect over a wider timescale from 30 min up to 180 min. Among the soy isoflavones (100 μM), genistein and daidzein, exhibited a slightly stronger inhibitory effect on glucose uptake than genistin and daidzin at each incubation time (Fig. 4). The maximum inhibition of glucose uptake by the four isoflavones occurred after 180 min.

Effect of soy isoflavones on insulin-stimulated 2-DG uptake in rat adipocytes

When the cells preincubated with the different soy isoflavones and then incubated with insulin at a physiological concentration, the insulin significantly stimulated glucose uptake into the adipocytes (Fig. 5, A). However, the actual insulin-stimulated glucose uptake was significantly suppressed by the aglycones, genistein (47.5%) and daidzein (24.8%), when compared to the vehicle control, yet not by the glycones, genistin and daidzin (Fig. 5, B).

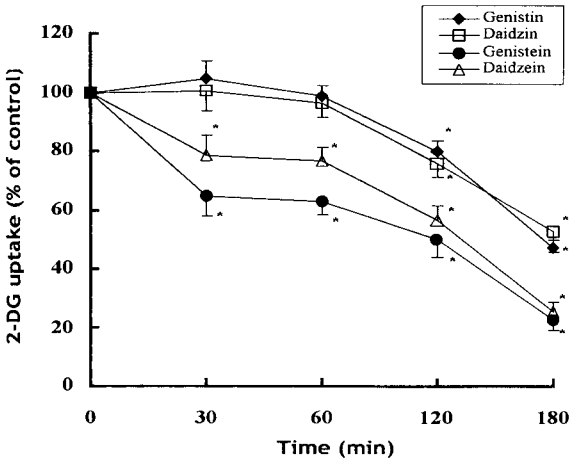

Fig. 4. Time dependence of isoflavone effects on [^{3}H]-2-DG uptake in isolated rat adipocytes. The cells were incubated with isoflavones (100 μM) in a KRH buffer at 37°C for 30 min, 60 min, 120 min, and 180 min, then further incubated for 3 min in the same buffer containing [^{3}H]-2-DG. The radioactivity incorporated into the cells was measured. Data are shown as mean ± SEM for five repetitions. *$p<0.05$ vs. control by Student's *t*-test.

Effect of soy isoflavones on 2-DG uptake and lipolysis in adipocytes isolated from high-fat fed rats

When the four isoflavones were added to the incubation mixture with the adipocytes isolated from rats fed a high-fat diet for 7~9 weeks, the glucose uptake was significantly inhibited (Fig. 6, A). The lipolysis activity was determined by the amount of glycerol released from the cells into the incubation medium, and found to be significantly stimulated by genistin and diadzin, and their aglycones (100 μM) when compared to the vehicle control (Fig. 6, B). However, genistein and daidzein stimulated more extensive lipolysis activity than genistin and daidzin, as indicated by the inhibited glucose uptake.

DISCUSSION

Evidence continues to emerge that soybeans and their components have a beneficial effect on lipid concentration in healthy and type 2 diabetic subjects. However, it is unclear whether this beneficial effect on lipids is due to the soy proteins, isoflavones, or fiber component. Vedavanam et al. (21) suggested that soy isoflavones may be beneficial for diabetic subjects because of their esterogenic activity and ability to prevent glucose-induced lipid peroxidation and inhibit intestinal glucose uptake by decreasing sodium-dependent glucose transport, resulting in a reduction in postprandial hyperglycemia. Isoflavones are one of the biologically active compounds in soybeans, and there are two types of soy isoflavone: glycones and aglycones. Accordingly, the aim of this study was to compare the effect of glycones and aglycones on glucose uptake and lipolysis in adipocytes isolated from high-fat fed rats.

The transport of glucose in adipose tissue plays a critical role in glucose homeostatsis: adipose-selective depletion of the insulin-responsive glucose transporter GLUT4 causes insulin resistance in mice (22). A major metabolic function of insulin is to increase the V_{max} of glucose uptake in muscle and adipose tissue (23,24). In this study, insulin caused an ~3-fold increase in glucose uptake compared to that in the control cells. However, the basal glucose uptake was inhibited 20~53% and 43~77% by the soy isoflavone glycones and aglycones, respectively, ($p<0.05$) in a concentration-dependent manner (25~100 μM). The soy isoflavones also signifi-

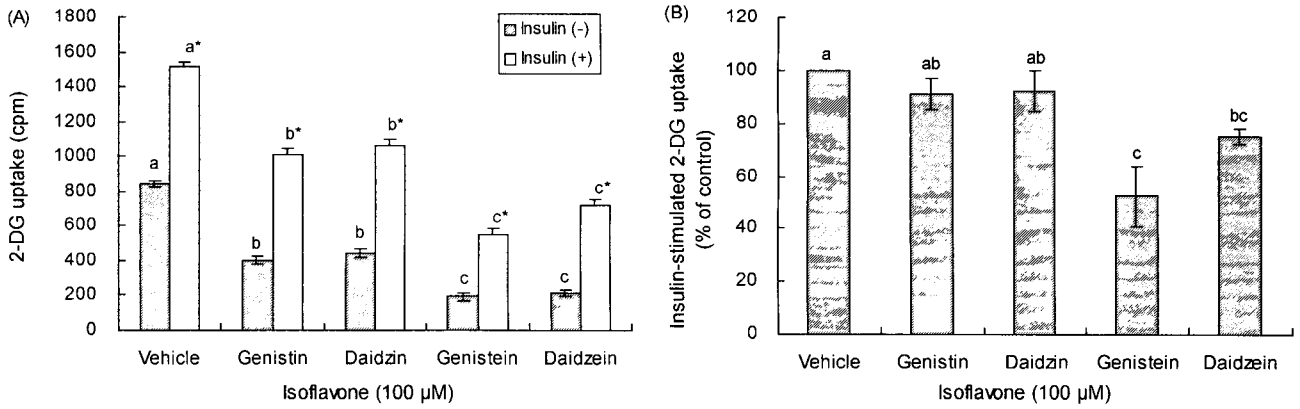

Fig. 5. Effect of isoflavones on insulin-stimulated [^{3}H]-2-DG uptake in isolated rat adipocytes. The cells were incubated with genistin, daidzin, genistein, and daidzein in a KRH buffer at 37°C for 3 hr, incubated without (-) or with (+) 1 nM insulin for 30 min before terminating the reaction, then further incubated for 3 min in the same buffer containing [^{3}H]-2-DG. The radioactivity incorporated into the cells was measured (A). The vehicle was incubated with DMSO. The insulin-stimulated [^{3}H]-2-DG uptake was calculated by subtracting the [^{3}H]-2-DG uptake in the absence of insulin from that in the presence of insulin (B). Data are shown as mean ± SEM for six repetitions. The means not sharing a common letter are significantly different between groups ($p<0.05$) by Duncan's multiple test. *$p<0.05$ vs. insulin (-) by Student's *t*-test.

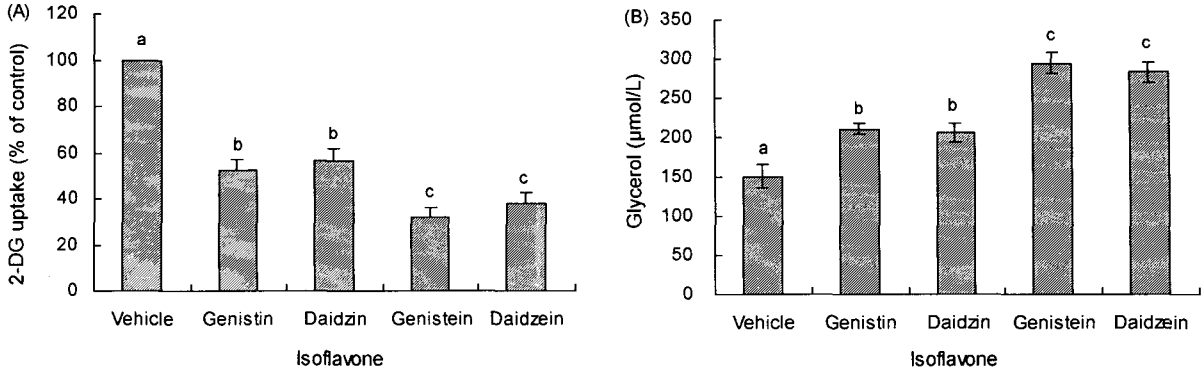

Fig. 6. Effect of isoflavones on [^{3}H]-2-DG uptake and lipolysis in adipocytes isolated from high-fat fed rats. After supplementing the rats with high-fat (50% of daily calorie intake) for 7~9 weeks, the adipocytes were isolated. The cells were incubated with genistin, daidzin, genistein, and daidzein in a KRH buffer at 37°C for 3 hr, then further incubated for 3 min in the same buffer containing [^{3}H]-2-DG. The radioactivity incorporated into the cells was measured. Data are shown as mean±SEM for ten repetitions (A). The medium used to incubate the rat adipocytes was also collected and assayed for the glycerol concentration, a measure of lipolysis (B). Data are shown as mean±SEM for five repetitions. The vehicle was incubated with DMSO. The means not sharing a common letter are significantly different between groups ($p<0.05$) by Duncan's multiple test.

cantly inhibited glucose uptake in the adipocytes isolated from high-fat fed rats, although the glucose uptake inhibiton was less than in adipoctyes from rats fed normal diets 3.4~12.5% (data not shown). Genistin and daidzin were less active than genistein and daidzein in inhibiting the glucose uptake in adipocytes isolated from both normal and high-fat fed rats. When insulin was incubated with genistin, daidzin, genistein, and daidzein, the glucose uptake was stimulated 2.5-, 2.4-, 2.8-, and 3.3-fold compared to the absence of insulin, however, the actual insulin-stimulated glucose uptake was significantly inhibited by 64% and 52% by genistein and daidzein, respectively, with genistein exhibiting a more potent effect than daidzein. In contrast, the glycones, genistin and daidzin, did not significantly inhibit the insulin-stimulated glucose uptake in the rat adipocytes. This may have been due to the loss of their polyphenolic character by *O*-glucoside formation (25). It was previously observed that genistein exerted an inhibitory effect on insulin-stimulated glucose uptake (13) and glucose metabolism (14) in white adipose tissue cells. Smith et al. (13) demonstrated that a genistein-induced inhibition of glucose uptake is due to conformational changes of GLUT4 without a significant drop in the number of membrane-associated glucose transports. Meanwhile, the present results showed that the inhibitory effect on glucose uptake by the genistein analogue, daidzein, was very similar to that by genistein.

White fat tissue cells, called adipocytes, play an important role in lipid synthesis, release, and storage in an organism. Triglycerides, which are the main form of lipids stored in adipocytes, are synthesized in these cells from glucose or fat taken up from the plasma. When triglycerides are broken down, glycerol and free fatty acids are released into the plasma. Thus, it is evident that the processes occurring in the adipocytes should be precisely regulated to avoid a fat excess or deficit in the overall organism. Hormonal regulation appears to be pivotal in maintaining the balance between lipid synthesis and breakdown in these cells. Under physiological conditions, epinephrine and glucagons augment lipolysis, whereas insulin exerts an antilipolytic action and increases lipogenesis (26). Accordingly, this study also examined the effect of soy isoflavones on lipolysis in adipocytes isolated from high-fat fed (50% of total calorie intake) rats. The lipolytic effect of genistein and daidzein was more potent than that of genistin and daidzin at 100 μM. Szkudelska et al. (27) previously reported that genistein at aconcentration of 0.1 mM and 1 mM significantly enhanced basal lipolysis in isolated rat adipocytes. In adipocytes, triglycerides are broken down by hormone sensitive lipase (HSL) in response to protein kinase A (PKA) preceded by an elevated cAMP content in the cells. Thus, lipolysis can be evoked by augmenting the cAMP content or as a result of the direct action of a lipolytic agent on PKA or HSL. In the present study, the soy isoflavones appeared to limit insulin action (28), especially genistein and daidzein, which clearly inhibited the glucose uptake and enhanced lipolysis in the isolated rat adipocytes.

In conclusion, the current results found that the inhibitory effect of daidzein on glucose uptake was very similar to that of genistein. Plus, the aglycone form was a more potent inhibitor of glucose uptake, while simultaneously a more potent stimulator of lipolysis than the glucoside form in the adipocytes isolated from high-fat fed rats. Thus, the present *in vitro* data would seem to indicate that regular consumption of genistin and daidzin,

and their aglycones may exacerbate insulin resistance in susceptible individuals via an impaired glucose uptake in adipose tissue. However, recently other studies reported that soy isoflavones improve lipid metabolism, produce an antidiabetic effect, and activate PPAR receptors *in vivo* (29,30). Accordingly, these *in vitro* results should not be used to predict the actual effects of soy isoflavones under *in vivo* conditions. Therefore, further *in vivo* studies of soy isoflavones are needed to validate their effects and elucidate their roles.

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REFERENCES

1. Heinonen SM, Hoikkala A, Wähälä K, Adlercreutz H. 2003. Metabolism of soy isoflavones daidzein, genistein and glycitein in human subjects. Identification of new metabolites having an intact isoflavonoid skeleton. *J Steroid Biochem Mol Biol* 87: 285-299.
2. Messina MJ, Persky V, Setchell KDR, Barnes S. 1994. Soy intake and cancer risk: a review of the *in vitro* and *in vivo* data. *Nutr Cancer* 21: 113-131.
3. Barnes S. 1998. Evolution of the health benefits of soy isoflavones. *Proc Soc Exp Biol Med* 217: 386-392.
4. Anthony MS, Clarkson TB, Williams JK. 1998. Effects of soy isoflavones on atherosclerosis: potential mechanism. *Am J Clin Nutr* 68: 1390S-1393S.
5. Hertog MG, Kromhout D, Aravanis C, Blackburn H, Buzine R, Fidanza F, Giampaoli, S, Jansen A, Menotti A, Nedeljkovic S. 1995. Flavonoid intake and long-term risk of coronary heart disease and cancer in the seven countries study. *Arch Int Med* 155: 381-386.
6. Chang YC, Nair MG. 1995. Metabolism of daidzein and genistein by intestinal bacteria. *J Nat Prod* 58: 1892-1896.
7. Chang YC, Nair MG, Nitiss JL. 1995. Metabolites of daidzein and genistein and their biological activities. *J Nat Prod* 58: 1901-1905.
8. Hawksworth G, Drasar BS, Hill MJ. 1971. Intestinal bacteria and the hydrolysis of glycosidic bonds. *J Med Microbiol* 4: 451-459.
9. Xu X, Duncan AM, Merz BE, Kurzer MS. 1998. Effects of soy isoflavones on estrogen and phytoestrogen metabolism in premenopausal women. *Cancer Epidemiol Biomarkers Prev* 7: 1101-1108.
10. Rowland I, Faughnan M, Hoey L, Wahala K, Williamson G, Cassidy A. 2003. Bioavailability of phyto-oestrogens. *Br J Nutr* 89: S45-S58.
11. Jayagopal V, Albertazzi P, Kilpatrick ES, Howarth EM, Jenningburns PE, Hepburn DA, Atkin SL. 2002. Beneficial effects of soy phytoesterogen intake in postmenopausal women with type 2 diabetes. *Diabetes Care* 25: 1709-1714.
12. Bhathena SJ, Valasquez MT. 2002. Beneficial role of dietary phytoesterogens in obesity and diabetes. *Am J Clin Nutr* 76: 1191-1201.
13. Smith RM, Tiesinga JJ, Shah N, Smith JA, Jarett L. 1993. Genistein inhibits insulin-stimulated glucose transport and decrease immunocytochemical labeling of GLUT4 carboxyl-terminus without affecting translocation of GLUT4 in isolated rat adipocytes: additional evidence of GLUT4 activation by insulin. *Arch Biochem Biophys* 300: 238-246.
14. Abler A, Smith JA, Randazzo PA, Rothenberg PL, Jarett L. 1992. Genistein differentially inhibits postreceptor effects of insulin in rat adipocytes without inhibiting the insulin receptor kinase. *J Biol Chem* 267: 3946-3951.
15. Nakashima S, Koike T, Nozawa Y. 1991. Genistein, a protein tyrosine kinase inhibitor, inhibits thromboxane A_2-mediated human platelet responses. *Mol Pharmacol* 39: 475-480.
16. Barnett M, Collier GR, Odea K. 1995. Energy intake with respect to the development of diabetes mellitus in psammomys obesus. *Diabetes Nutr Metab* 8: 42-47.
17. Riccardi G, Giacco R, Rivellese AA. 2004. Dietary fat, insulin sensitivity and the metabolic syndrome. *Clin Nutr* 23: 447-456.
18. Chalkley SM, Hettiarachchi M, Chisholm DJ, Kraege EW. 2002. Long-term high-fat feeding leads to severe insulin resistance but not diabetes in Wistar rats. *Am J Physiol Endocrinol Metab* 282: E1231-E1238.
19. Rodbell M. 1964. Metabolism of isolated fat cells; effects of hormones on glucose metabolism and lipolysis. *J Biology Chem* 239: 375-380.
20. Olefsky JM. 1978. Mechanisms of ability of insulin to activate the glucose-transport system in rat adipocytes. *Biochem J* 172: 137-145.
21. Vedavanam K, Srijayanta S, O'Reilly J, Raman A, Wiseman H. 1999. Antioxidant action and potential antidiabetic properties of an isoflavonoid-containing soyabean phytochemical extract (SPE). *Phytother Res* 13: 601-608.
22. Baumann CA, Saltiel AR. 2003. Spatial compartmentalization of signal transduction in insulin action. *Bioessays* 23: 215-222.
23. Crofford OB, Renold AE. 1965. Glucose uptake by incubated rat epididymal adipose tissue. Characteristics of the glucose transport system and action of insulin. *J Biol Chem* 240: 3237-3244.
24. Simpson IA, Cushman SW. 1986. Hormonal regulation of mammalian glucose transport. *Annu Rev Biochem* 55: 1059-1089.
25. Kapiotis A, Hermann M, Held I, Seelos C, Ehringer H, Gemeiner BMK. 1997. Genistein, the dietary-derived angiogenesis inhibitor, prevents LDL oxidation and protects endothelial cells from damage by atherogenic LDL. *Arterioscler Thromb Vasc Biol* 17: 2868-2874.
26. Szkudelski T, Szkudelski K. 2000. Glucose as agent; studies on isolated rat adipocytes. *Physiol Res* 49: 213-217.
27. Szkudelska K, Nogowski L, Szkudelski T. 2000. Genistein affects lipogenesis and lipolysis in isolated rat adipocytes. *J Steroid Biochem Mol Biol* 75: 265-271.
28. Akiyama T, Ishida J, Nakagawa S, Ogawara H, Watanabe S, Itoh N, Shibuya M, Fukami Y. 1987. Genistein, a specific inhibitor of tyrosine-specific protein kinase. *J Biol Chem* 262: 5592-5595.
29. Mezei O, Banz WJ, Steger RW, Peluso MR, Winters TA, Shay N. 2003. Soy isoflavones exert antidiabetic and hypolipidemic effects through the PPAR pathways in obese zucker rats and murine RAW 264.7 cells. *J Nutr* 133: 1238-1243.
30. Kim S, Shon I, Lee YS, Lee YS. 2005. Hepatic gene expression profiles are altered by genistein supplementation in mice with diet-induced obesity. *J Nutr* 135: 33-41.

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