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β-Conglycinin Embeds Active Peptides That Inhibit Lipid Accumulation in 3T3-L1 Adipocytes in Vitro

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Obesity is a worldwide health concern because it is a well-recognized predictor of premature mortality. The objective was to identify soybean varieties that have improved potential to inhibit fat accumulation in adipocytes by testing the effects of soy hydrolysates having a range of protein subunit compositions on lipid accumulation and adiponectin expression in 3T3-L1 adipocytes. The results showed that differences in the protein distribution of 15 soy genotypes led to different potentials for the reduction of fat accumulation. The inhibition of lipid accumulation of soy alcalase hydrolysates in 3T3-L1 adipocytes ranged from 29 to 46%. Soy hydrolysates made from genotypes with 45.3 ± 3.3% of total protein as β-conglycinin, on average, showed significantly higher inhibition of lipid accumulation compared to those with 24.7 ± 1.5% of extracted total protein as β-conglycinin. Moreover, after in vitro simulated digestion with pepsin−pancreatin of the soy alcalase hydrolysates, 86% of the original activity remained. Adiponectin expression was induced in 3T3-L1 adipocytes treated with 15 soy hydrolysates up to 2.49- and 2.63-fold for high and low molecular weight adiponectin, respectively. The inhibition of lipid accumulation calculated from a partial least squares (PLS) analysis model correlated well with experimental data ($R^2 = 0.91$). In conclusion, it was feasible to differentiate soy varieties on the basis of the potential of their proteins to reduce fat accumulation using a statistical model and a cell-based assay in vitro. Furthermore, β-conglycinin embeds more peptides than glycinin subunits that inhibit lipid accumulation and induce adiponectin in 3T3-L1 adipocytes. Therefore, soy ingredients containing β-conglycinin may be important food components for the control of lipid accumulation in adipose tissue.

KEYWORDS: β-Conglycinin; glycinin; soy peptides; alcalase hydrolysis; 3T3-L1 adipocytes

INTRODUCTION

Obesity is a worldwide health concern because it is a well-recognized independent predictor of premature mortality (*1*). It is also a complex metabolic disorder that is thought to result from an imbalance of energy intake and energy expenditure leading to the excess accumulation of fat in various adipose tissues and organs (*2*). Moreover, it often coexists with insulin and leptin resistance, diabetes, high blood pressure, thrombosis, cardiovascular disease, and osteoporosis (*3, 4*). The rising prevalence of obesity and obesity-related diseases and treatment complications has exacerbated health-care cost inflation (*5*).

Recent studies showed that adipocyte dysfunction plays an important role in the development of obesity (*6*). Adipocytes synthesize and secrete biologically active molecules called adipocytokines. Adiponectin is one of the most important adipocytokines and is specifically and highly expressed in adipocytes (*7*). Adiponectin is secreted primarily as an active high molecular weight (HMW) complex and a low molecular weight (LMW) complex. The HMW complex is closely associated with the known physiological and metabolic effects of adiponectin (*8*). The plasma adiponectin concentration and mRNA expression level are decreased in obese individuals (*9*−*12*). The administration of adiponectin improves insulin action accompanied by increases in fatty acid oxidation and a decreased triacylglycerol level in muscle (*13*). At the cellular level, obesity involves two different physiological components, lipid metabolism and adipogenesis (*14*). Lipid metabolism is the energy flow into or out of adipocytes (lipogenesis and lipolysis, respectively). Adipogenesis, is the discernible cellular transition through which a spindle-shaped fibroblastic cell proceeds, first forming a preadipocyte, then a multilocular adipocyte and, finally, a mature (unilocular) adipocyte (*14*). Research continues to focus on the

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role of diet, specifically dietary protein, as a preventive strategy that may be beneficial for the management of obesity.

Soybean provides one of the most abundant plant sources of dietary protein (*15*). The protein content of soybeans varies from 39 to 45% (*16, 17*). The major protein components of soybeans are seed storage proteins known as β-conglycinin and glycinin, which account for 50−70% of total seed proteins (*18, 19*). β-Conglycinin is a trimer with a molecular mass of 150−200 kDa; it is composed of three subunits, α, α′, and β (*20*). Glycinin is a hexamer with 320−375 kDa and with five major subunits A1aB2, A2B1a, A1bB1b, A5A4B3, and A3B4. Each subunit consists of an acidic chain (about 40 kDa) and a basic chain (about 20 kDa), joined by disulfide bonds (*21*).

A number of studies suggest that consumption of soy protein has favorable effects on obesity by suppression of food intake and increased satiety and/or energy expenditure that may reduce body fat and weight (*22−25*). There is a suggestive body of evidence indicating that soy foods may confer additional benefits promoting other metabolic consequences (*26*). The specific soy components that may cause these metabolic improvements are not known and it will require more extensive experimentation. The objective of this study was to identify and predict soybean varieties that have improved potential to inhibit fat accumulation in adipocytes based on the statistical correlation between soybean protein composition and inhibition of lipid accumulation and adiponectin expression in 3T3-L1 adipocytes.

MATERIALS AND METHODS

Chemicals. Alcalase from *Bacillus licheniformis* (EC 3.4.21.62), pepsin (EC 3.4.23.1, 662 units/mg, enzyme/flour 1:20 w/w, pH 2.0), pancreatin (8× USP, from porcine pancreas, enzyme/flour 1:20 w/w, pH 7.5), isobutylmethylxanthine (IBMX), dexamethasone (DEX), insulin, 100 mM sodium pyruvate solution, penicillin (1000 units/mL), and streptomycin (1000 units/mL) were purchased from Sigma-Aldrich (St. Louis, MO). Dulbecco's modified Eagle's medium (DMEM) and sodium pyruvate were from Gibco-BRL (Rockville, MD). Fetal bovine serum (FBS) was from PAA Laboratories GmbH (Linz, Austria). Anti-adiponectin (Acrp30) goat polyclonal IgG antibody (Santa Cruz Biotechnology, Santa Cruz, CA) was raised against a peptide mapping with an internal region of adiponectin of human origin recommended for detection of precursor and mature adiponectin of mouse, rat and human origin. Anti-actin mouse monoclonal IgG and bovine anti-goat IgG horseradish peroxidase conjugates were purchased from Santa Cruz Biotechnology. ECL anti-mouse IgG horseradish peroxidase conjugates was purchased from GE Healthcare (Piscataway, NJ).

Materials. Fifteen soybean samples were provided by the Monsanto Co. (St. Louis, MO) (*27*). β-Conglycinin and glycinin were purified in our laboratory as described previously (*28*). Nonfat dry milk (NFDM) was purchased from Nestle S.A. (Vevey, Switzerland). 3T3-L1 (also designated ATCC CCL-92.1) fibroblasts from Swiss albino mouse were purchased from the American Type Culture Collection (Rockville, MD).

Preparation of Defatted Soy Flour. Soybean samples (150 g) were ground using a Thomas-Wiley model 4 mill (Thomas-Wiley, Swedesboro, NJ) with a 2 mm screen. Ground materials were then reground in the same mill using a 1 mm (S1−S6) or 2 mm (S7−S15) sieve. Samples were extracted using hexane with traditional Soxhlet extractor. Aliquots of approximately 25 g were extracted for a minimum of 5 h. Extracted samples were air-dried for 2−3 h, and samples from the same soybeans were combined.

Preparation of Soy Protein Hydrolysates. Briefly, 2 g of defatted flour, NFDM, purified β-conglycinin or glycinin were added to 25 mL of deionized water and brought to 50 °C at pH 8.0. Then, 5 mg of alcalase (11 units/mg) was added. Hydrolysis was carried out for 3 h at 50 °C and pH 8.0 was maintained by adding 0.5 M NaOH. Hydrolysis was stopped by the addition of 75 μL of 0.1 N HCl. Hydrolysates were centrifuged at 14000*g* at 10 °C for 30 min. After centrifugation, 10% trichloroacetic acid (TCA) was added in a 1:1 ratio. Once TCA was added, the hydrolysates were centrifuged again under the same conditions, and the liquid hydrolysates were filtered using stirred ultrafiltration cell 1 kDa membrane (Millipore). The peptides were freeze-dried in a FreeZone freeze-dry system (Kansas City, MO) and kept at −80 °C. Protein DC assay (Bio-Rad) was followed for protein quantification using a standard curve using bovine serum albumin (BSA) ($y = 0.0002x - 0.0021$, $R^2 = 0.997$).

Alcalase hydrolysates were further hydrolyzed with pepsin−pancreatin to mimic gastrointestinal digestion following the method described by Wang et al. (*28*).

SDS-PAGE of Soy Flours and Soy Hydrolysates. Ground soy flours were extracted with 1× Laemmli SDS buffer pH 6.8 with 0.07 M DTT (~30 mg/mL). For each sample, 10 μg of total protein was run on a 26 lane 12% homogeneous Bis-Tris Criterion gel. A broad-range prestained SDS-PAGE standard (Bio-Rad Laboratories, Hercules, CA) was used as molecular weight marker. The gels were stained in Colloidal Coomassie Blue G-250, destained in distilled water, and imaged using the GS 800 Calibrated Densitometer (Bio-Rad Laboratories). Protein quantitation was performed using Bio-Rad Quantity One Software. The software was used to determine the relative quantity of each band in the sample lane. Proteins were reported as the relative percent of the total protein bands in the lane. Protein bands were identified comparing the experimental molecular mass with the theoretical molecular mass calculated from the amino acid sequence of the proteins given by the ProtParam program (http://ca.expasy.org/tools/protparam.html) (**Table 1**). Furthermore, the identities of the major protein bands of the 15 soybean genotypes, on the SDS-PAGE gel, were compared with previous publications (*28−30*).

Soy hydrolysates were diluted with tricine sample buffer (Bio-Rad Laboratories) with 2% β-mercaptoethanol and boiled for 4 min prior to loading. The peptide bands of extracted hydrolysates were analyzed by SDS-PAGE using PhastGel Gradient 8−25% ready gels from GE Healthcare (Waukesha, WI). A prestained precision Plus Protein standard (Bio-Rad Laboratories) was used. The gel was run at 250 V, 10 mA, 3.0 W, 15 °C, and 99 Vh and fixed for 30 min in fixing solution (40% methanol, 10% acetic acid). Then the gel was stained with Coomassie Blue G-250 overnight, destained with 10% acetic acid, and imaged using a Kodak Image Station 440 CF (Eastman Kodak Co., New Haven, CT).

Isoflavone and Saponin Analysis. To determine isoflavone and saponin concentrations, the 15 soybean flours were extracted with dimethyl sulfoxide/methanol (1:1) solution at room temperature for 4 h, followed by a 15 min sonication at 50 °C and another 1−2 h of extraction at room temperature. The extracts were then filtered through

Table 1. Calculated Molecular Masses of Major Soy Proteins[a]

	name	no. of aa	mol mass (Da)
β-conglycinin	α′ subunit	577	67240.0
	α subunit	543	63151.8
	β subunit	414	47776.5
glycinin	G1 precursor	495	**55706.3**
	A1a chain	287	32646.9
	Bx chain	180	19955.5
	G2 precursor	485	**54390.7**
	A2 chain	278	31622.8
	B1a chain	180	19773.2
	G3 precursor	481	**54241.7**
	A chain	275	31483.7
	B chain	180	19911.4
	G4 precursor	562	**63587.1**
	A5 chain	97	10540.8
	A4 chain	257	29953.9
	B3 chain	185	20743.5
	G5 precursor	516	**57956.1**
	A3 chain	320	36392.4
	B4 chain	172	19049.5

[a] Amino acid sequences of major soy proteins were retrieved from UniProtKB/Swiss-Prot Release 56.1 of Sept 1, 2008, and the theoretical molecular mass of each protein was calculated using the ProtParam program (http://ca.expasy.org/tools/protparam.html).

a 0.45 μM nylon 66 filter for both isoflavone and saponin analysis. The HPLC analysis was conducted on a Hewlett-Packard series 1100 HPLC system equipped with an Inertsil ODS-3 reverse phase C-18 column (5 μm, 250 × 4.6 mm, with a Metaguard column, from Varian) and a G1316A column oven. The system was controlled by HPChem Station version A.06.01. For isoflavone analysis, a linear water−methanol gradient from 20 to 100% in 53 min was used, with 0.025% trifluoroacetic acid (TFA) added to both solvents. The flow rate was set at 1 mL/min, and the effluent was monitored at 260 nm. Isoflavone concentration was calculated by using the standard curves of pure standards of daidzein, glycitein, and genistein. As the molar extinction coefficients are essentially the same for all forms of the individual soy isoflavones (aglycone, glucoside, acetyl glucoside, and malonyl glucoside), the use of the molar extinction coefficients from the glucoside forms gives an accurate quantitative determination (*31*). This means that pure aglycone or glucoside standards for each isoflavone can be used to quantitate all of the glycoside forms on a molar extinction basis. The molecular weight values for each of the 12 forms of isoflavones found in soy was used in the calculations to convert the peak area to micrograms of isoflavones injected to determine the most accurate concentrations [peak area (mAbs) × molar extinction coefficient (nM/mAbs) × MW of the isoflavone species (μg/nM) = μg of isoflavone injected]. This value is then converted into milligrams per gram of sample (μg in sample/μL injected × μL total sample volume/mg of sample extracted).

For saponin analysis, a linear water−acetonitrile gradient from 30 to 50% in 45 min was used, with 0.025% TFA added to both solvents. The flow rate was set at 1 mL/min, and the effluent was monitored at 210 nm. Saponin concentrations were calculated by using standard curves prepared from a characterized mix of group B saponins prepared in the laboratory. The extinction coefficient for soyasaponin I was used to quantitate the group A saponins and the 2,3-dihydro-2,5-dihydroxy-6-methyl-4*H*-pyran-4-one (DDMP) for conjugated B group saponins. Identification of isoflavone and saponin peaks was confirmed by comparison with standards and/or LC-MS analysis (*32*).

Chymotrypsin Inhibitory Units (CTIU) Determination. The concentrations of total chymotrypsin inhibitors in soybean flours were measured as CTIU by following published procedures (*33*). Briefly, soybean flours were extracted at pH 9.5−9.8 using dilute sodium hydroxide. A known volume of chymotrypsin solution was mixed with the sample suspension or with a sample blank, to measure the base level of protein in the reaction mixtures. Casein was then added as the substrate for chymotrypsin hydrolysis. The hydrolysis was stopped at exactly 10 min by adding trichloroacetic acid. The amount of chymotrypsin inhibitor was calculated from the decrease in trichloroacetic acid soluble protein, as determined by absorbance at 275 nm with a UV spectrophotometer. One chymotrypsin inhibition unit is defined as a decrease of 0.01 absorbance unit at 275 nm in a 1 cm path length cell, per 10 mL of final reaction volume after 10 min of reaction.

Cell Culture and Treatments. The 3T3-L1 preadipocytes were seeded at 6×10^3 cells/cm^2 in 6-well plates and cultured in DMEM containing 10 mM sodium pyruvate, 100 units/mL penicillin, 100 units/mL streptomycin, and 10% FBS (FBS/DMEM medium). For induction of preadipocyte differentiation, 2 days after reaching 100% confluence, the cells were stimulated with FBS/DMEM medium containing 167 nM insulin, 0.5 M IBMX, and 1 M DEX for 2 days. Cells were then maintained in FBS/DMEM medium with 167 nM insulin for another 2 days, followed by culturing with FBS/DMEM medium for an additional 4 days, at which time up to 90% of cells were mature adipocytes with accumulated fat droplets.

Mature adipocytes were treated separately with soy, purified β-conglycinin, and glycinin alcalase hydrolysates dissolved in water at a concentration of 100 μM (on the basis of dose−response preliminary data). This concentration corresponds to 1 mg of soluble protein/mL of medium, and it was calculated using an average molecular mass of 10 kDa determined in this study. Adipocytes were incubated at 37 °C in a 5% CO_2 atmosphere for 72 h for determination of lipid accumulation and cell viability and for 24 h for detection of adiponectin expression.

3T3-L1 Adipocytes Cytotoxicity Assay. For the cytotoxicity assay, 3T3-L1 preadipocytes were seeded (1.5×10^4 cells/cm^2) in 96-well flat-bottom cell culture plates and differentiated and further treated as mentioned above. The CellTiter 96AQueous One Solution was used to determine the number of viable cells according to the manufacturer's manual (Promega, Madison, WI). Briefly, the CellTiter 96AQueous One Solution (20 μL) was added to 100 μL of medium-containing wells (with cells), and then the plate was incubated in a 5% CO_2 incubator at 37 °C. After 2 h, absorbance was measured at 515 nm with a 96-well plate reader (Biotek Instruments, Winooksi, VT). Cell viability percentages were calculated using the following equation:

$$A_{\text{treatment,515nm}} / A_{\text{control,515nm}} \times 100 = \%\ \text{cell viability}$$

Lipid Quantification in 3T3-L1 Adipocytes by Oil Red O Assay. Briefly, treated adipocytes were washed with Dulbecco's phosphate-buffered saline (DPBS) and fixed with 10% formalin (in DPBS) in 6-well plates for 1 h. Then, cells were washed with 60% isopropanol and allowed to air-dry. The Oil Red O stock solution (6:4 v/v with water) was added to lipid droplets for 10 min. After Oil Red O lipid staining, cells were washed with water four times and were air-dried. Oil Red O dye was eluted by adding 100% isopropanol after 10 min of incubation at room temperature. OD_{510nm} of eluted isopropanol was measured using a CytoFluor Series 4000 multiwell luminescence plate reader (PerSeptive Biosystems Inc., Framingham, MA). Inhibition percentages of lipid accumulation were calculated using the following equation:

$$(A_{\text{control,510nm}} - A_{\text{treatment,510nm}})/A_{\text{control,510nm}} \times 100 = \%\ \text{inhibition of lipid content}$$

Adiponectin Expression by Western Blotting. Treated adipocytes were lysed in sample loading buffer (Laemmli buffer containing 5% 2-mercaptoethanol) and sonicated three times using an ultrasonic cell disruptor from Misonix Inc. (Farmingdale, NY). Protein content of the cell lysates was determined using DC protein assay from Bio-Rad Laboratories following the instructions of the manufacturer. Samples were loaded on an equal protein basis of 25 μg/lane. Samples were subjected to SDS-PAGE using 4−20% Tris-HCl SDS-PAGE ready gels. Gels were run through a mini-electrophoresis kit at 200 V constant for 30 min. Furthermore, proteins were transferred to a polyvinylidene difluoride (PVDF) membrane (Bio-Rad) in transfer buffer (25 mM Tris, pH 8.3, 192 mM glycine, and 0.1% SDS) using Western sandwich assembly for 1 h at 4 °C using 125 V. After the transfer, the membrane was blocked with 5% nonfat dry milk (NFDM) in Tris-buffered saline containing 0.1% Tween 20 (TBST) for 1 h, followed by an overnight incubation with anti-adiponectin goat polyclonal IgG antibody (1:200) or anti-actin mouse monoclonal IgG (1:200) at 4 °C. Furthermore, the membrane was washed with TBST four times and was incubated with bovine anti-goat IgG horseradish peroxidase conjugates (1:1000) or ECL anti-mouse IgG horseradish peroxidase conjugates (1:1000), respectively, for 1 h at room temperature. The membrane was washed again in TBST four times, and signals were visualized using chemiluminescence (Amersham Bioscience) and a Kodak Image Station 440 CF (Eastman Kodak Co.).

Partial Least-Squares (PLS) Regression Analysis. PLS regression analyses were carried out using the proc PLS procedure of SAS version 9 (SAS Institute, Cary, NC) to evaluate the relationship between bioactive soy components (*X* variables, including matrix protein profile, concentrations of total isoflavones, total saponins in soy hydrolysates) and either the inhibition (percent) of lipid accumulation or adiponectin expression values of the 15 soy hydrolysates (SH1−SH15) on 3T3-L1 adipocytes (*Y* variable). A regression model, $Y = XWQ + E$ (where *X* is the dependent-variable matrix; *W* is the weight matrix for *X*; *Q* is the loading matrix for the *Y* variable; and *E* is the error term), was developed to predict in vitro the potential of soy components to inhibit lipid accumulation, to induce adiponectin, and to evaluate the contribution of each component on these activities. Because soy protein hydrolysates contain numerous peptides, it is not possible to consider the contribution of each one, and most likely, peptides work together through additive and synergistic ways; therefore isolating one or two peptides has its inherent limitations (*34*). For this reason, we considered the matrix protein profile instead.

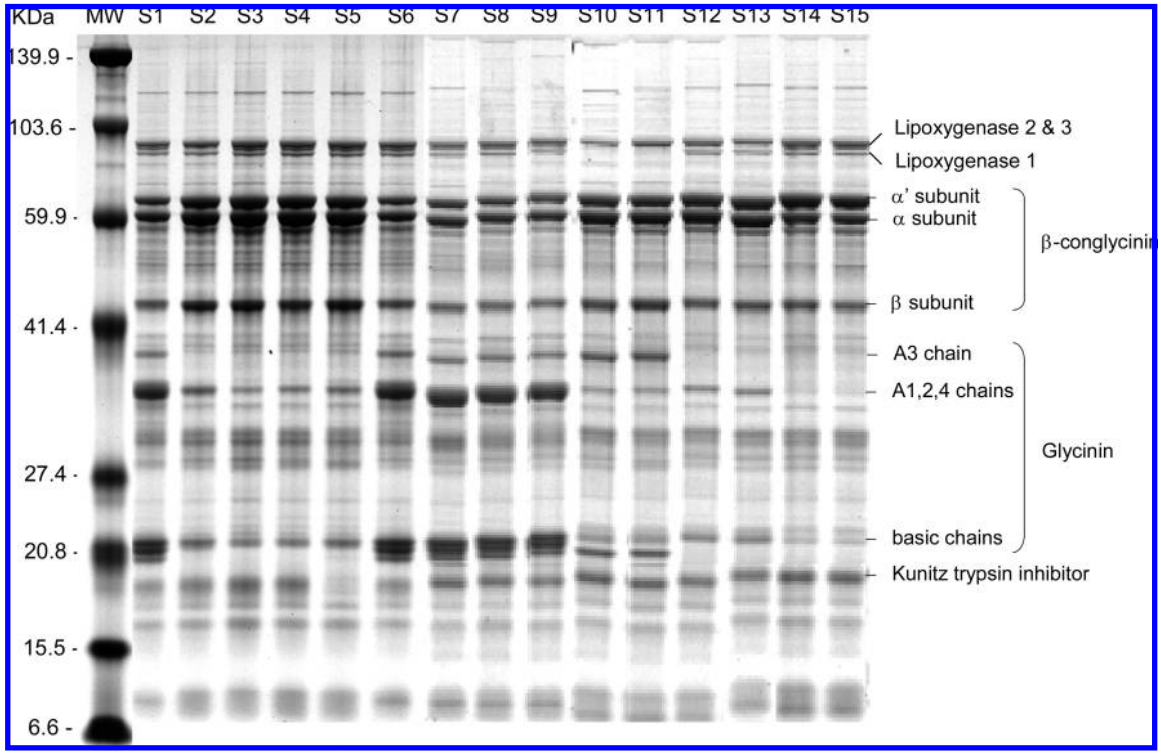

Figure 1. Protein profiles of soy flours from 15 soy genotypes (S1−S15). For each sample, 10 μg of total protein was run on a 26 lane 12% homogeneous Bis-Tris Criterion gel. The molecular mass markers (lane 1) consisted of myosin (193,916 Da), β-galactosidase (103,624 Da), bovine serum albumin (59,883 Da), ovoalbumin (41,374 Da), carbonic anhydrase (27,354 Da), soybean trypsin inhibitor (20,778 Da), lysozyme (15,505 Da), and aprotinin (6,598 Da).

Other Statistical Analyses. The concentration to inhibit 50% of 3T3-L1 adipocyte viability (IC_{50}) was determined by nonlinear regression (curve fit) using GraphPad Prism software. The difference between samples was evaluated by using one-way ANOVA with LSD (least-squares differences) test, and $p < 0.05$ was considered to be significant. One-way ANOVA analysis was run by the GLM procedure of SAS (SAS Institute, Cary, NC).

RESULTS AND DISCUSSION

Protein Profile of 15 Soy Genotypes. The total protein concentration of S1−S15 ranged from 42.4 to 48.2% (data not shown). Protein extracts of different soy genotypes (S1−S15) showed different protein profiles (**Figure 1**). SDS-PAGE showed nine major protein bands corresponding to lipoxygenase isoforms 1 (92.9 kDa), 2 and 3 (89.1 kDa), β-conglycinin subunits (α, α', and β) ranging from 67 to 45 kDa, glycinin subunits (A1, 2, 3, 4 and basic chains) ranging from 38.5 to 20.5 kDa, and Kunitz trypsin inhibitor (18.6 kDa). The identities of these protein bands agreed with other publications (*28*−*30*). Soy genotypes S1, S6, S7, S8, and S9 had 24.7 ± 1.48% of extracted protein as β-conglycinin subunits and 35.3 ± 3.3% of extracted protein as glycinin subunits, whereas soy genotypes S2−S5 and S10−S15 had 45.3 ± 0.5% of extracted protein as β-conglycinin subunits and 6.3 ± 4.9% of extracted protein as glycinin subunits.

Table 2 shows the percent contribution of each identified protein to total proteins of defatted soy flour from different genotypes S1−S15. Considerable variation among the 15 soy genotypes was observed for the α' subunit (8.1−20.7% total protein), α subunit (10.4−22.2% total protein), and β subunit (4.9−12.9% total protein) of β-conglycinin. Similarly, the protein percentage distribution of A3 chain (0.0−5.6% total protein), A1,2,4 chains (0.0−15.9% total protein), and basic (0.0−17.7% total protein) glycinin subunits varied among soy genotypes. S14 and S15 were completely lacking glycinin subunits. The total β-conglycinin and total glycinin content of the 15 genotypes ranged from 23.1 to 50.8% of total protein and from 0.0 to 35.7% of total protein, respectively. ANOVA analysis divided 15 genotypes into 2 groups, a 45% BC group (S2−S5 and S10−S15 with average β-conglycinin 45.3 ± 3.3% of total extracted protein) and a 25% BC group (S1 and S6−S9 with average β-conglycinin 24.7 ± 1.5% of total extracted protein).

The subunit composition of glycinin among soybean cultivars has been investigated by Natarajan et al. (*35*). They reported considerable variation in all 5 glycinin subunits among 16 soybean genotypes including wild, ancestors of North American cultivars, landraces from Asia, and modern genotypes using proteomic and genetic analysis. Cai and Chang (*36*) showed total glycinin values from 36.3 to 51.1% of total protein by SDS-PAGE among 13 soybean cultivars from North Dakota, which are higher than our results. The 45% BC group was a result of a selective breeding program for lower glycinin or for the absence of glycinin, such as in lines S14 and S15. These changes may have health benefits because β-conglycinin has been proposed to have a potential role in obesity by lowering the accumulation of fat in the liver, suppressing food intake, and causing gastric emptying in rats (*22, 37, 38*). There is typically an inverse relationship between β-conglycinin and glycinin concentrations. Some plant breeders have taken advantage of the relationship by developing new lines high in β-conglycinin or glycinin for different purposes.

Lipoxygenases isoforms 2 plus 3 and 1 and Kunitz trypsin inhibitor were also found in soy genotypes to have different levels of abundance, ranging from 3.2 to 7.2% of total protein, from 0.0 to 2.4% of total protein, and from 1.5 to 7.5% of total protein, respectively.

Table 2 also summarizes isoflavone and saponin concentrations in defatted soy from different genotypes (S1−S15). The

Table 2. Protein Percentage Distribution As Determined by SDS-PAGE, Total Isoflavones, and Total Saponins of Defatted Soy Flour from Different Genotypes (S1−S15)[a]

compd	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15
							Percent Total Proteins								
lipoxygenases 2 and 3	4.9ef	5.3ed	6.3bc	6.9ab	7.0ab	5.9cd	4.0g	5.1e	5.0e	3.2h	5.2de	5.5de	4.2fg	7.2a	6.9ab
lipoxygenase 1	1.7cd	2.0b	1.9b	2.4a	2.0b	1.8bc	1.2e	1.6d	1.3e	0.0f	0.0f	2.3a	1.6cd	1.9b	1.8bc
α′ subunit of β-conglycinin	8.7f	15.1cde	16.1bc	15.1cde	15.7cd	8.3f	8.2f	8.1f	8.2f	14.1de	13.5e	18.0b	17.7b	20.7a	20.1a
α subunit of β-conglycinin	11.9efg	20.4abc	20.8ab	20.8ab	22.2a	11.6fg	11.1fg	10.4g	10.4g	18.6dc	17.7d	20.8ab	20.0bc	13.7e	13.1ef
β subunit of β-conglycinin	5.6ef	12.4a	10.4b	10.5d	12.9a	6.3e	5.2fg	4.9fg	4.5g	8.3cd	11.3b	7.7d	9.2c	8.6cd	7.6d
glycinin A3 chain	2.6c	0.0d	0.0d	0.0d	0.0d	2.9bc	3.1b	3.1bc	2.9bc	5.6a	5.5a	0.0d	0.0d	0.0d	0.0d
glycinin A1,2,4 chains	15.9a	3.8c	1.8e	2.0e	2.6ed	14.9b	15.3ab	14.6b	14.8b	2.1e	1.8e	2.6d	3.0d	0.0f	0.0f
glycinin basic chains	17.1a	3.9c	2.7c	2.4c	3.3c	16.7a	17.2a	17.5a	17.7a	7.0b	6.5b	3.5c	3.0c	0.0d	0.0d
Kunitz trypsin inhibitor	3.7ed	4.7cd	5.2bc	4.8bcd	1.5f	3.3e	5.5bc	5.1bc	5.1bc	7.1a	5.9b	5.9b	5.6bc	7.5a	7.5a
total β-conglycinin	26.2d	**48.0ab**	**47.2b**	**46.5b**	**50.8a**	26.2d	24.5d	23.4d	23.1d	**40.9c**	**42.5c**	**46.5b**	**46.9b**	**43.0c**	**40.8c**
total glycinin	**35.6a**	7.8c	4.5d	4.4d	5.9cd	**34.5a**	**35.7a**	**35.3a**	**35.4a**	14.6b	13.7b	6.1cd	6.0cd	0.0e	0.0e
							Milligrams per Gram of Flour								
total isoflavones	1.66f	2.07f	2.11f	6.62a	5.00cd	4.79d	3.46e	3.52e	3.89e	5.36c	6.37ab	6.28ab	6.05b	6.01b	5.35c
total saponins	4.66	3.25	3.16	1.20	0.80	1.66	7.87	7.86	9.27	9.24	8.85	7.02	8.25	9.45	9.90
							CTIU (Dry Basis)								
chymotrypsin inhibitor	11	20	20	25	20	14	17.5	13.6	14.8	23.4	16.9	20.2	21.1	27.9	29.1

[a] Means with different letters in the same row are significantly different ($p < 0.0001$).

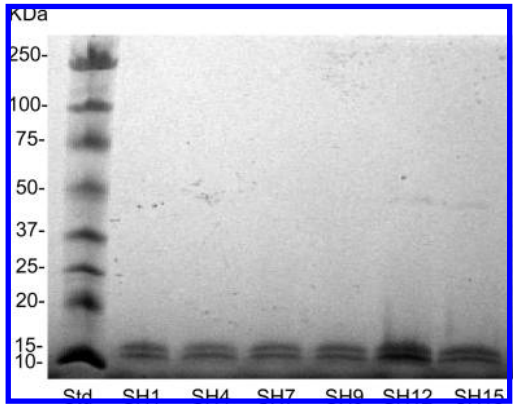

Figure 2. Protein profile of soy after enzymatic hydrolysis with alcalase at 50 °C for 3 h by SDS-PAGE. For each sample, 10 μg of total protein was run on 8−25% gradient Phast-gel (GE Healthcare). Precision Plus Protein standard was included as molecular weight marker (lane 1).

isoflavone and saponin concentrations in soy genotypes ranged from 1.66 to 6.62 mg/g of flour and from 0.80 to 9.9 mg/g of flour, respectively. The amount of isoflavones in soybean varies depending upon the type of soybean, geographic area of cultivation, and harvest years (*39*). Total isoflavone content in defatted soy flour has been reported from 73 to 168 mg/100 g of edible portion (*40*), which is lower than the values reported in the present work. The molar extinction coefficients of all four forms of the individual soy isoflavones (aglycone, glucoside, acetyl glucoside, and malonyl glucoside) are essentially the same. This is generally true for most ester-substituted phenolic compounds, such as the flavonoids (*41, 42*) and the overlapping spectra of three forms of genistein (*31*).

Soya saponins are one of the major classes of phytochemicals present in soy. Saponin concentrations in defatted soy flours were lower than other reported values, which were 0.5−2% (*39*) and 0.6−6.2% (*32*) of dry weight depending on cultivar and growing conditions (*43*).

Figure 2 shows the protein profile of soy after enzymatic hydrolysis with alcalase at 50 °C for 3 h. The molecular masses of proteins in the nonhydrolyzed defatted soy flour ranged from 10 to 93 kDa (**Figure 1**), whereas after 3 h of alcalase hydrolysis all proteins larger than 15 kDa were digested into smaller proteins and peptides at the molecular masses of 14 and 10 kDa to even smaller peptides, which are believed to have diffused out of the gel. The difference in protein profiles of each soy genotype shown in the present work may provide different relative amounts of peptides after enzymatic hydrolysis, and as a result variations in the antiobesity potential of soy hydrolysates may occur.

By comparison of the values for total isoflavone and saponin concentrations in defatted soy flours with their hydrolyzed counterparts, average reductions of 53 ± 18 and 94 ± 2% in isoflavone and saponin concentrations, respectively, were found (data not shown). Moreover, aglycone forms of isoflavones (genistein, glycitein, and daizdein), known to be biologically active, were not detected in soy protein hydrolysates. Therefore, saponin and isoflavone values were not considered as variables to include in the PLS model because concentrations were substantially reduced in the 15 soy protein hydrolysates. Isoflavone and saponin losses occurred during the preparation of soy protein hydrolysates including centrifugation, and the final ultrafiltration step using 1000 Da of MWCO membranes, led to reductions in the concentration of these compounds.

Effect of Soy Hydrolysates on Lipid Accumulation in 3T3-L1 Adipocytes. Figure 3A presents the percentage of inhibition of lipid accumulation by Oil Red O assay in 3T3-L1 adipocytes after 72 h of treatment with 100 μM soy protein hydrolysates (SH1−SH15) compared to their control. Treatment of 3T3-L1 adipocytes with 100 μM soy protein hydrolysates for 72 h inhibited cell growth from 10 to 19% (data not shown). This is typical as long as 80% viability is guaranteed. These values were taken into account to correct the percentage of inhibition of lipid accumulation. Treatment of soy hydrolysates at a concentration of 100 μM decreased lipid accumulation in 3T3-L1 adipocytes compared to untreated cells. Different soy hydrolysate samples showed different activities, SH2−SH5 showing the highest inhibition of lipid accumulation (39−46%, inhibition), followed by SH10−SH15 (33−37% inhibition). SH1, SH6, SH7, SH8, and SH9 exhibited the lowest reduction of lipid content, ranging from 27 to 30%, although no statistically significant differences were found between the last two sets of samples.

Figure 3B presents the average inhibition of lipid content of both the 25% BC (SH1 and SH6−SH9) and 45% BC group

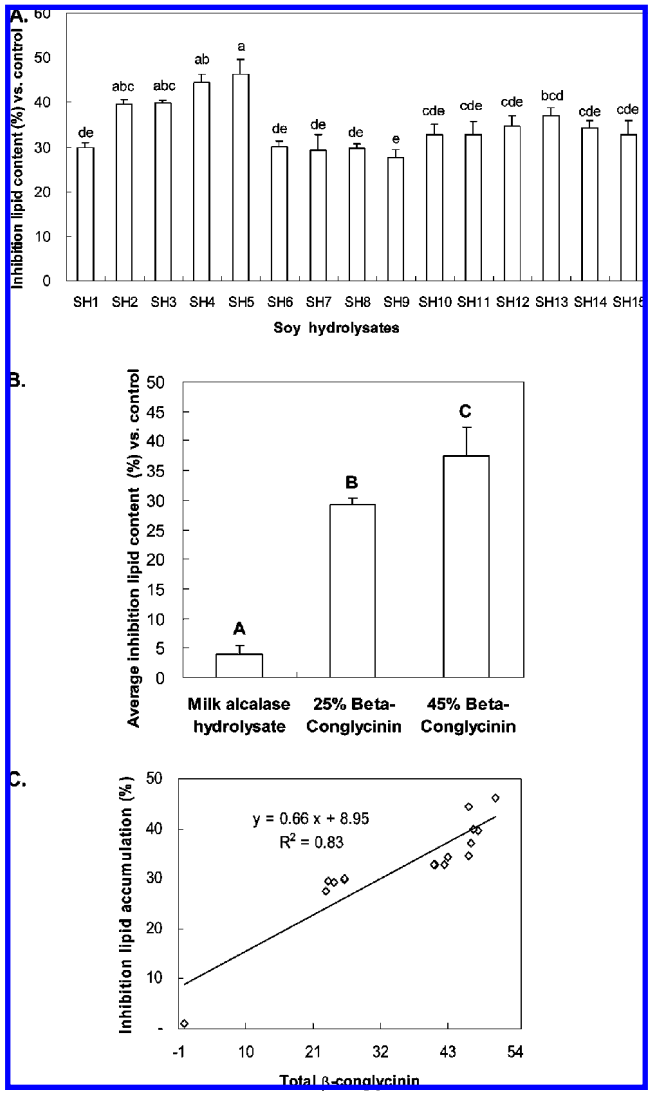

Figure 3. (**A**) Effect of soy alcalase hydrolysates (SH1−SH15) on inhibition of lipid accumulation (percent) versus control in 3T3-L1 adipocytes. 3T3-L1 adipocytes were harvested 8 days after the initiation of differentiation. Cells were treated with 100 μM soy hydrolysates for 72 h at 37 °C in a humidified 5% CO_2 incubator. (**B**) Comparison between average inhibition of lipid accumulation values (percent) of milk alcalase hydrolysate and SHs derived from proteins containing 24.7 ± 1.5% (S1, S6, S7, S8, and S9) and 45.3 ± 3.3% β-conglycinin (S2−S5 and S10−S15). Error bars indicate the standard deviation. Different letters indicate significant difference, $p < 0.0001$. (**C**) Correlation between percent inhibition of lipid accumulation and total β-conglycinin content of soy (and control) alcalase hydrolysates.

(SH2−SH5 and SH10−SH15). The average inhibition of lipid content of the 45% BC group (37.5%) was significantly higher ($p < 0.0001$) than that of the 25% BC group (29.3%), indicating that β-conglycinin embeds more active peptides with an effect on adipocyte lipid accumulation. To compare the inhibitory effect of soy hydrolysates in lipid accumulation in 3T3-L1 adipocytes with another high-quality protein source, milk alcalase hydrolysate was tested. The latter showed a low reduction in the lipid content (4% inhibition) of 3T3-L1 adipocytes treated during 72 h. Despite the fact that cell viability of adipocytes treated with milk protein hydrolysates was 90%, milk hydrolysates did not have any effect on lipid accumulation, demonstrating the better potential of soy alcalase hydrolysates for reduction of lipid accumulation in 3T3-L1 adipocytes. Higher total β-conglycinin soy varieties correlated ($R^2 = 0.83$) with higher percentage of inhibition of lipid accumulation (**Figure 3C**). A control with no soy prepared under the same conditions of soy hydrolysates was tested, showing no effect on inhibition of lipid accumulation in 3T3-L1 adipocytes (**Figure 3C**). Furthermore, the effect of in vitro simulated gastrointestinal digestion on inhibition of lipid accumulation in 3T3-L1 adipocytes was studied. Results showed that after pepsin−pancreatin digestion of soy alcalase hydrolysates an average of 86.13 ± 10.69% of the original activity remained.

The present study showed that soy protein hydrolysates exert an inhibitory effect on lipid accumulation in 3T3-L1 adipocytes. This suggests that soy protein alcalase hydrolysates somehow affected lipid metabolism (lipogenesis and/or lipolysis) in 3T3-L1 adipocytes. This possibility is supported by observations from several other studies with soy protein isolates. Nawasaga et al. (*44*) reported that soy protein isolate lowered the triglyceride (TG) content and fatty acid synthase mRNA level in adipose tissue. Tovar et al. (*45*) concluded that soy protein may limit adiposity by reducing the number of dysfunctional adipocytes, possibly as a result of low lipogenesis in obese Zucher fa/fa rats. Torre-Villalvazo et al. (*46*) reported that brown adipose tissue from rats fed a high-fat diet containing soy protein oxidized fatty acids to a higher extent than brown adipose tissue from rats fed a high-fat diet containing casein and, as a consequence, fatty acid storage and lipid droplet area decreased.

Several in vivo studies have focused on the role of β-conglycinin in lipid metabolism. A randomized double-blind placebo-controlled study in 138 volunteers aged 26 to 69 years showed that the intake of 5 g of β-conglycinin per day decreased visceral fat (*38*). Moreover, it has been reported in mice and rats that feeding β-conglycinin lowered fatty acid synthesis, increased the activities of β-oxidation enzymes such as carnitine palmitoyl transferase and acyl-coA oxidase in the liver, and increased fecal excretion of TG (*37, 47*).

Effect of Soy Hydrolysates on Adiponectin Expression in 3T3-L1 Adipocytes. Panels **A** and **B** of **Figure 4** show, respectively, the fold increase in HMW and LMW adiponectin expression, in 3T3-L1 adipocytes after treatment for 24 h with 100 μM soy protein hydrolysates (SH1−SH15). **Figure 4C** presents the detection of adiponectin and actin in adipocytes by Western blotting. HMW adiponectin induction was detected in 3T3-L1 adipocytes treated with soy hydrolysates SH4, SH5, and SH7−SH14 at a concentration of 100 μM. SH4, SH5, and SH11 showed the highest induction of adiponectin ($p < 0.0001$) (2.49-, 2.71-, and 2.27-fold increase, respectively) compared to the control. Similarly, LMW adiponectin was induced when cells were treated with soy hydrolysates SH3−SH5 and SH7−SH15 at a concentration of 100 μM. SH5 and SH10−SH13 exhibited significantly ($p < 0.0001$) higher induction of LMW adiponectin levels (2.63-, 2.73-, 2.43-, 2.43-, and 2.57-fold increase, respectively) compared to the control.

Total adiponectin induction values of SHs derived from proteins containing >40% β-conglycinin (S2−S5 and S10−S15) showed a tendency to stimulate higher total adiponectin expression (3.05-fold increase vs control) than SHs derived from proteins containing <26% β-conglycinin (S1, S6, S7, S8, and S9) (2.37-fold increase vs control); however, both groups were not statistically different from each other. More research is needed to understand the compositional factors responsible for the differences observed.

Adiponectin has been shown to play a role in regulating adipocytes differentiation and secretory function and in enhancing insulin sensitivity (*48*). Plasma levels of this hormone are reduced in obesity (*10*). There are several in vivo studies

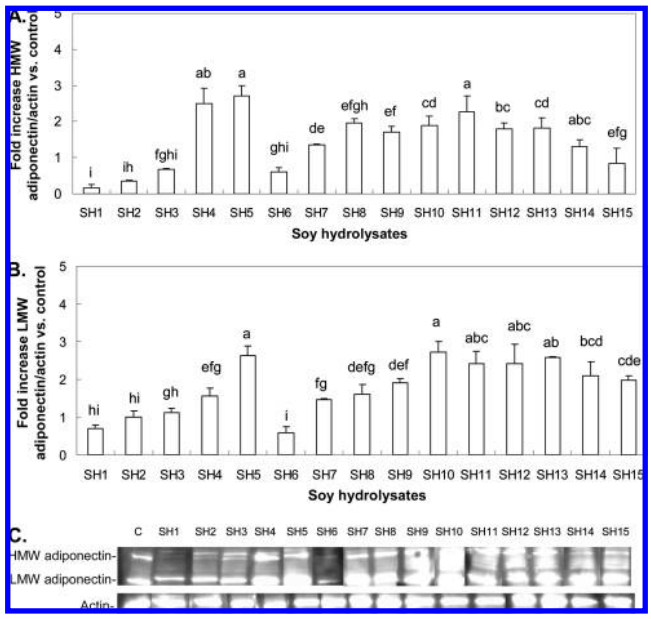

Figure 4. Induction of adiponectin levels in 3T3-L1 adipocytes after treatment for 24 h with 100 μM soy protein hydrolysates (SH1−SH15): (**A**) fold increase in high molecular weight (HMW) adiponectin (60 kDa) and (**B**) low molecular weight (LMW) adiponectin (30 kDa) of SHs compared to their controls. The relative expression of adiponectin in 3T3-L1 adipocytes was quantified densitometrically and calculated according to the reference bands of β-actin. (**C**) Detection of adiponectin and actin levels in 3T3-L1 adipocyte lysates by Western blot.

showing that soy protein may modulate adiponectin production (*44*). The action of adiponectin has, to some degree, been characterized in the liver and in skeletal muscle cells, but its effect on adipocytes is still largely unknown. In several clinical and mice studies, adiponectin was also found in liver and muscle cells to stimulate 5′-adenosine monophosphate (AMP)-activated protein kinase (AMPK) and enhance lipid oxidation, increase insulin sensitivity, which improves glucose metabolism (inhibiting glucose production by liver), and decrease plasma triglycerides and blood glucose and body weight (*49, 50*). Because some studies have found AMPK in adipocytes, it is possible that adiponectin may have similar effects in adipose tissue (*51*). In mice, it was found that adiponectin decreases body weight by stimulating energy expenditure (*52*). Spranger et al. (*53*) found no adiponectin in human cerebrospinal fluid and no evidence of secretion of adiponectin in the brain, which implied that the energy expenditure by adiponectin was caused by an indirect mechanism.

Kinetic studies in our laboratory have shown that optimum times to detect changes in adiponectin protein expression and in lipid accumulation are 24 and 72 h, respectively (*54*). These results agree with the approach used by other investigators who, as in our study, also measured lipid accumulation in 3T3-L1 adipocytes after 72 h of treatment (*55, 56*). Although bioactive peptides must probably exert their physiological effect at the moment they enter into contact with target tissues, longer times are needed to detect changes in protein expression and even more to detect further changes in cell function such as lipid accumulation.

PLS Regression Analysis. To further understand the correlation between bioactive components in soy protein and the in vitro inhibition of lipid accumulation of soy protein hydrolysates in 3T3-L1 adipocytes, PLS regression analyses were conducted using the proteins listed in **Table 2** as variables.

Table 3. Estimated Partial Least Squares (PLS) Weight of First (Inhibition of Lipid Accumulation) and Second Variables (Protein Content), Regression Coefficients (*B*), and Variable Importance to Projection (VIP)[a]

variable	PLS regression coefficient (*B*)	VIP
α subunit of β-conglycinin	0.30	**1.29**
β subunit of β-conglycinin	0.29	**1.29**
α′ subunit of β-conglycinin	0.09	**0.94**
lipoxygenase 1	0.09	0.74
lipoxygenases 2 and 3	0.11	0.63
Kunitz trypin inhibitor	−0.29	0.92
glycinin A3 chain	−0.12	0.90
glycinin basic chains	−0.11	1.07
glycinin A1,2,4 chains	−0.10	0.99

[a] Regression coefficients (*B*) were calculated to obtain an overall statistical evaluation of the contribution of each variable to the final activity. The higher the absolute *B* value is, the higher the impact, whereas the sign indicates positive ("+") or negative ("−") effect on inhibition of lipid accumulation value. Variable importance for projection (VIP) of Wold was calculated to summarize each variable contribution to fit the PLS model for both variables and response. A VIP value of <0.9 (Wold, 1995) was considered to be small, indicating the variable was not important to the model.

Unlike linear regression, PLS does not give a simple regression formula. The regression model can be built in SAS and indicates the importance of each soy component on the inhibition of lipid accumulation and total adiponectin induction in 3T3-L1 adipocytes. To obtain an overall statistical evaluation of the contribution of each variable to the final activity, regression coefficients (*B*) were calculated from weights of the first (protein content) and second variables (lipid accumulation or total adiponectin induction). The higher the absolute *B* value, the higher the impact on inhibition of lipid accumulation or total adiponectin induction. The sign indicates a positive ("+") or negative ("−") effect on inhibition percentage of lipid accumulation or total fold increase of adiponectin versus control values. The variable importance for projection (VIP) of Wold was also calculated to summarize the contribution of each variable to fit the PLS model for both variables and responses (*57*). A VIP value of <0.9 was considered to be small, indicating the variable was not important to the model (*58*). **Table 3** presents the *B* and VIP values for each protein variable, on the inhibition of lipid accumulation, arranged by positive or negative *B* values and then sorted by VIP values. Of nine variables considered in the model, five had a positive *B* value. This meant that higher concentrations of these five variables led to higher inhibition of lipid accumulation in 3T3-L1 adipocytes. On the other hand, there were four variables that showed a negative *B* value, indicating a negative correlation with inhibition of lipid accumulation in 3T3-L1 adipocytes. All β-conglycinin subunits α ($B = 0.30$, VIP = 1.29), β ($B = 0.29$, VIP = 1.29), and α′ ($B = 0.09$, VIP = 0.94) showed a VIP of >0.9 and a positive regression coefficient, indicating a positive correlation with inhibition of lipid accumulation in 3T3-L1 adipocytes. Conversely, all of the major glycinin chains, A3 chain ($B = -0.12$, VIP = 0.90), basic chains ($B = -0.12$, VIP = 1.07), A1, 2, and 4 chains ($B = -0.11$, VIP = 0.99), and Kunitz trypsin inhibitor ($B = -0.29$, VIP = 0.92) showed a large VIP value and a negative regression coefficient. Kunitz was included as an example of protease inhibitors.

Lipoxygenase 1 ($B = 0.09$) and lipoxygenases 2 plus 3 ($B = 0.11$) exhibited VIP values of <0.90, indicating those variables were not important to the model. These results also indicated

Table 4. Estimated Partial Least Squares (PLS) Weight of First (Total Adiponectin Induction) and Second Variables (Protein Content), Regression Coefficients (*B*), and Variable Importance to Projection (VIP)[a]

variable	PLS regression coefficient (*B*)	VIP
α subunit of β-conglycinin	0.15	**1.18**
α′ subunit of β-conglycinin	0.09	**1.01**
β subunit of β-conglycinin	0.07	**0.92**
glycinin A3 chain	0.13	0.87
Kunitz trypsin inhibitor	0.04	0.44
lipoxygenases 2 and 3	−0.21	1.23
glycinin A1,2,4 chains	−0.16	1.35
glycinin basic chains	−0.09	1.08
lipoxygenase 1	−0.04	0.51

[a] Regression coefficients (*B*) were calculated to obtain an overall statistical evaluation of the contribution of each variable to the final activity. The higher the absolute *B* value is, the higher the impact, whereas the sign indicates positive ("+") or negative ("−") effect on total adiponectin induction value. Variable importance for projection (VIP) of Wold was calculated to summarize each variable contribution to fit the PLS model for both variables and response. A VIP value of <0.9 (Wold, 1995) was considered to be small, indicating the variable was not important to the model.

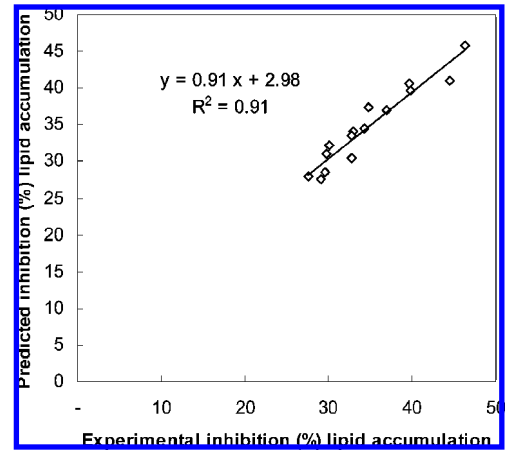

Figure 5. Correlation between experimental and predicted inhibition of lipid accumulation values of soy hydrolysates on 3T3-L1 adipocytes. A PLS model was built to calculate predicted lipid accumulation inhibition values from the protein distribution (percent total protein) in soy hydrolysates.

that β-conglycinin embeds more active peptides that inhibit lipid accumulation in 3T3-L1 adipocytes than glycinin.

Table 4 presents the *B* and VIP values for each protein variable, on adiponectin induction, arranged by positive or negative *B* values and then sorted by VIP values. Of nine variables considered in the model, five had a positive *B* value. This meant that higher concentrations of these 5 variables led to higher total adiponectin induction in 3T3-L1 adipocytes. On the other hand, there were four variables that showed a negative *B* value, indicating a negative correlation with total adiponectin induction in 3T3-L1 adipocytes. All β-conglycinin subunits α ($B = 0.15$, VIP = 1.18), α′ ($B = 0.09$, VIP = 1.01), and β ($B = 0.07$, VIP = 0.92) showed a VIP of >0.9 and a positive regression coefficient, indicating a positive correlation with total adiponectin induction in 3T3-L1 adipocytes. In contrast, lipoxygenases 2 and 3 ($B = -0.21$, VIP = 1.23) and all of the major glycinin chains such as A1, 2, and 4 chains ($B = -0.16$, VIP = 1.35) and basic chains ($B = -0.09$, VIP = 1.08) showed a VIP of >0.9 and a negative regression coefficient. Glycinin A3 chain ($B = 0.13$) and lipoxygenase 1 ($B = -0.04$) exhibited VIP values of <0.9, indicating those variables were not important to the model.

Moreover, a predicted value can be calculated from the PLS model. **Figure 5** is an example showing a good correlation between the predicted lipid accumulation inhibition values calculated by the PLS regression model and the experimental values ($R^2 = 0.91$).

The PLS regression study suggested that the matrix protein composition was important to cellular processes that have the potential to reduce lipid accumulation. β-Conglycinin embedded more active peptides that induced inhibition of lipid accumulation and total adiponectin expression in 3T3-L1 adipocytes than glycinin. To confirm the contributions of β-conglycinin and glycinin hydrolysates, purified samples of these proteins were tested.

Effect of Pure β-Conglycinin and Glycinin on Lipid Accumulation and Adiponectin Expression in 3T3-L1 Adipocytes. To confirm that β-conglycinin embeds more active peptides that inhibit lipid accumulation than glycinin, purified β-conglycinin and glycinin were hydrolyzed with alcalase following the same procedure as for defatted soy flours. **Figure 6A** shows the dose-dependent cytotoxicities of β-conglycinin and glycinin alcalase hydrolysates on 3T3-L1 adipocytes, and the IC_{50} values were found at 490 and 980 μM, respectively, after 72 h treatment. **Figure 6B** shows a dose-dependent inhibition of lipid accumulation versus control in 3T3-L1 adipocytes after 72 h of exposure to pure β-conglycinin and glycinin alcalase hydrolysates. At concentrations of 10 and 100 μM, β-conglycinin hydrolysates showed significantly higher inhibition ($p < 0.0001$) of lipid accumulation (5.1 and 44.8% inhibition, respectively) than glycinin hydrolysates (−8.0 and −18.5% inhibition, respectively), confirming the previous PLS findings. **Figure 6C** shows adiponectin induction (fold increase versus control) in 3T3-L1 adipocytes after 24 h of exposure to 100 μM pure β-conglycinin and glycinin alcalase hydrolysates. The results showed that 3T3-L1 adipocytes treated with β-conglycinin hydrolysates exhibited significantly ($p < 0.0001$) higher LMW and HMW adiponectin expression (1.64- and 2.36-fold increase vs control, respectively) compared to glycinin alcalase hydrolysates (0.72- and 0.74-fold increase vs control, respectively).

Certain subunits of β-conglycinin have been shown to have an important role in obesity management. In Sprague−Dawley rats, oral administration of the soybean β-conglycinin peptone suppresses food intake and gastric emptying (*59*). Similarly, in rats fed a hypercholesterolemic diet, ingestion of the α′ subunit of β-conglycinin produced substantial reductions in plasma lipids as well as a marked up-regulation of liver β-VLDL receptors (*60*). A soybean β-conglycinin diet was also shown to lower serum triglyceride glucose and insulin levels in normal and genetically obese (KK-Ay) mice (*37*). These effects were accompanied by reduced hepatic fatty acid synthase activity and increased activities of two enzymes related to fatty acid β-oxidation and mRNA of acyl-CoA oxidase levels, as well as increased fecal excretion of triglycerides.

Conclusions. The objective of this study was to identify soybean varieties that have improved potential to control fat accumulation in adipocytes while also serving as a source of nutrients to maintain muscle mass. Progress toward this goal was made by developing a statistical model to predict the in vitro effects on adipocytes of soy hydrolysates on the basis of the correlation between the protein composition of their nonhydrolyzed counterparts and the inhibition of lipid accumulation and adiponectin induction in 3T3-L1 adipocytes.

The results showed that 15 soy genotypes exhibited different protein distribution and isoflavone and saponin

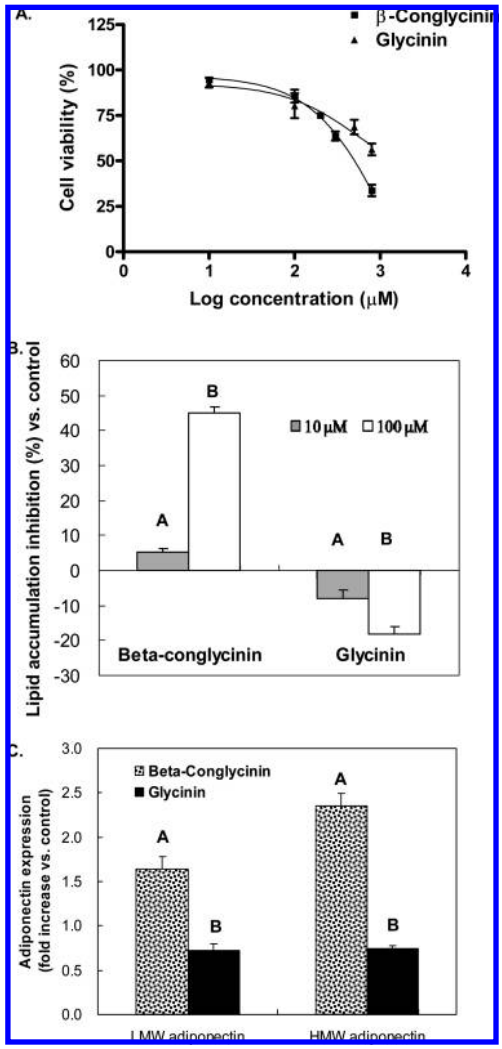

Figure 6. (**A**) Dose-dependent 3T3-L1 adipocyte cell viability after 72 h of exposure to pure β-conglycinin and glycinin alcalase hydrolysate. The results are expressed as percent viability of treated cells compared with the untreated control. (**B**) Dose-dependent inhibition of lipid accumulation (percent) versus control in 3T3-L1 adipocytes after 72 h of exposure to pure β-conglycinin and glycinin alcalase hydrolysate. (**C**) Dose-dependent induction of HMW and LMW adiponectin in 3T3-L1 adipocytes after 24 h of treatment with pure β-conglycinin and glycinin alcalase hydrolysates. Error bars indicate standard deviation.

concentrations. After alcalase hydrolysis, isoflavones and saponins were substantially reduced. Protein composition differences in defatted soy flour led to different levels of inhibition of lipid accumulation, with SH5 showing the highest inhibition of lipid accumulation and the highest induction of adiponectin expression. Thus, the present approach shows that it is feasible to identify soybean varieties suitable for their commercial exploitation as value-added products from soybean for weight management. The correlation study indicated that the matrix protein composition played an important role in the prevention of lipid accumulation in adipocytes. In this regard, β-conglycinin subunits embed more active peptides than glycinin subunits to reduce the lipid content and induce adiponectin expression in 3T3-L1 adipocytes. Therefore, β-conglycinin might be an important food component for the control of lipid accumulation in adipose tissue.

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