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Presence of Lunasin in Plasma of Men after Soy Protein Consumption

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Lunasin is a 43-amino acid bioactive peptide from soybean and other plant sources which is reported to possess anti-inflammatory and anticancer properties. The objective of this study was to assess the presence and concentration of lunasin in blood of men fed soy protein products. Five healthy male subjects who were 18−25 years old consumed 50 g of soy protein for 5 days, and blood was taken 30 min and 1 h after soy protein ingestion on day 5. Lunasin was isolated from plasma using strong anion exchange beads in a magnetic particle concentrator and eluted with 20 mM triethanolamine at pH 8.0 with 0.20 M NaCl. The concentration of lunasin in plasma as determined by an enzyme-linked immunosorbent assay ranged in the various subjects from 50.2 to 110.6 ng/mL of plasma (average ± standard deviation, 66.0 ± 25.4 ng/mL) for blood taken at 30 min and from 33.5 to 122.7 ng/mL of plasma (71.0 ± 32.8 ng/mL) for blood withdrawn 1 h after ingestion on day 5. We estimated an average of 4.5% absorption (range of 2.2−7.8%) of lunasin from the total lunasin ingested from 50 g of soy protein. Matrix-assisted laser desorption ionization time-of-flight peptide mass mapping showed that a 5 kDa peptide similar to synthetic lunasin was present in plasma samples of people who consumed soy protein while absent at the baseline plasma samples from the same individuals. Liquid chromatography−tandem mass spectrometry analysis showed the presence of amino acid sequences from lunasin in plasma samples after soy intake for 30 min and 1 h. No peptides from lunasin were present in plasma samples without soy intake. The results of this study suggest that lunasin is bioavailable in humans, an important requirement for its anticancer potential.

KEYWORDS: Soybean; lunasin; bioavailability; plasma; absorption

INTRODUCTION

Soybean is an important source of food protein not only because it provides most of the essential amino acids but also because it contains naturally occurring peptides with biological activities (*1*). It has also received an increasing amount of public interest due to its reported health benefits attributed to its bioactive components which include isoflavones, saponins, peptides, and proteins. On average, soybean seeds contain 40% protein conformed by different protein types. Seed storage proteins known as β-conglycinin and glycinin are the major components of soy proteins and account for 50−70% of total seed proteins (*2*, *3*). Biologically active peptides and proteins from soybean can be naturally present or derived by enzymatic hydrolysis or fermentation. For example, enzymatic hydrolysis of glycinin and β-conglycinin has resulted in the production of bioactive peptides with antihypertensive (*4*, *5*), hypocholesterolemic (*6*), and immunostimulating (*7*) properties. In addition, soybean contains naturally occurring biologically active proteins and peptides such as Bowman-Birk inhibitor (BBI) (*8*), lectin (*9*), Kunitz inhibitor (*10*), and the novel peptide lunasin (*11*). A comprehensive review of soy peptides and the different techniques used in their analysis has been recently published (*1*).

Lunasin is a novel bioactive peptide, originally isolated from soybean, with demonstrated anticancer properties in experimental animals (*12*, *13*). It is composed of 43 amino acids with nine aspartic acid residues on its carboxyl end. Its anticancer effect at the cellular level has been attributed to its ability to modify chromatin (*13*). Nevertheless, for a potential anticancer effect in humans, a key question is whether lunasin, a peptide with a relatively high molecular weight, is absorbed by the gastrointestinal system. A recent study in experimental animals showed that lunasin can be found in the liver and blood of rats fed lunasin-enriched soybean protein (*14*). However, this question has not been addressed in humans. The objective of this study was therefore to identify the presence and quantity

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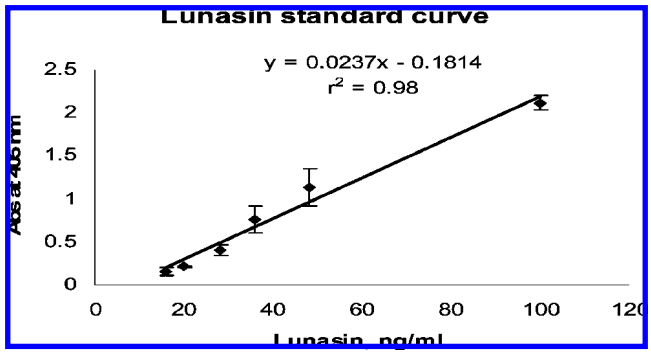

Figure 1. Standard curve of synthetic lunasin from an ELISA.

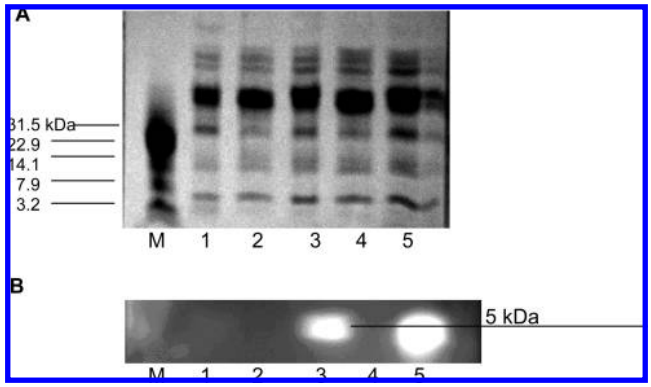

Figure 2. Gel electrophoresis (**A**) and Western blot (**B**) of synthetic lunasin spiked to baseline plasma. Lane M contained molecular mass markers for peptides, lane 1 baseline plasma, lanes 2 and 4 baseline plasma spiked with 5 μg of synthetic lunasin eluted with 20 mM triethanolamine with 0.15 M NaCl, and lanes 3 and 5 baseline plasma spiked with 10 μg of synthetic lunasin eluted with 20 mM triethanolamine with 0.15 M NaCl.

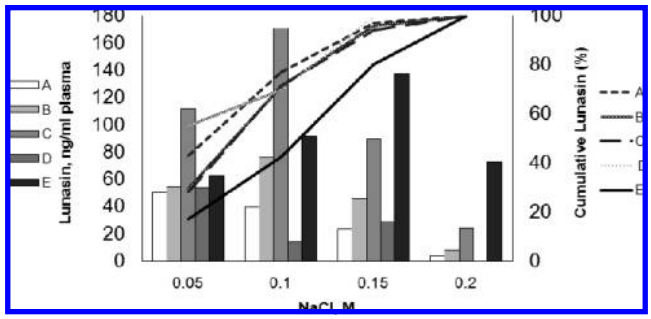

Figure 3. Lunasin concentration (nanograms per milliliter) and cumulative lunasin (percentage) in plasma samples from men (A−E) eluted sequentially from SAX beads with different concentrations of NaCl in 20 mM triethanolamine.

of lunasin in human plasma after consumption of soy protein, using ion exchange chromatography, mass spectrometry, and an enzyme-linked immunosorbent assay (ELISA).

MATERIALS AND METHODS

Materials. The soy protein came from two sources, a high-protein soybean isolated powder (90% protein) used to prepare soymilk (Genisoy Food Co., Tulsa, OK) obtained from the local market and a whole soybean chili dish prepared at the National Research Soybean Laboratory (University of Illinois) using canned soybeans (Eden organic canned black beans). Each serving of these foods provided 25 g of soy protein. Lunasin mouse monoclonal antibody was produced against the lunasin epitope (EKHIMEKIQGRGDDDDD) at the University of California (Berkeley, CA). Dynal magnet concentrator and Dynabeads SAX were purchased from Invitrogen Dynal (Oslo, Norway). Synthetic lunasin was obtained from American Peptides (Sunnyvale, CA).

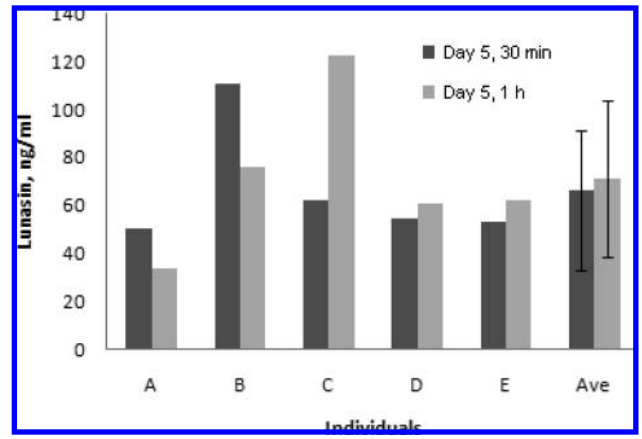

Figure 4. Lunasin concentrations in plasma from five human subjects 30 min and 1 h after intake of 50 g of soy protein for 5 days: (black bars) amount of lunasin in plasma 30 min after intake and (gray bars) amount of lunasin in plasma 1 h after intake.

Human Feeding Trial. The research protocol was approved by the Institutional Review Board of the University of Illinois. A written informed consent form was signed by all participants. Five healthy Caucasian males who were 18−25 years old were recruited for the study. Exclusion parameters included smoking, vegetarianism, and taking dietary supplements. The study lasted for two weeks. Week 1 was a washed out period in which participants were asked to avoid consumption of soy products. They were given a list of soy-containing products and food establishments that they should avoid such as Asian restaurants that offered soy foods. In week 2, participants consumed daily for lunch for a period of 5 days 50 g of soy protein provided by a soy milk shake containing 25 g of soy protein and a soy chili dish containing 25 g of soy protein. Both foods were consumed simultaneously. Blood samples were collected from each participant, immediately before (baseline) and at the end of the feeding period (day 5), in vacutainer tubes at the McKinley Health Clinic, University of Illinois, by a registered medical technologist. Blood samples collected at the end of the study (day 5) were obtained 30 min and 1 h after soy product consumption. Approximately 15 mL of blood was withdrawn from each individual per time of blood collection.

Preparation of Blood Samples. Collected blood samples were transported from the university clinic to the laboratory in racks within a leak proof container. Blood samples were centrifuged immediately at 3000*g* for 15 min at 4 °C to separate the plasma from the blood cells. Plasma samples were then stored at −80 °C until they were used.

Isolation of Lunasin from Human Plasma. Dynal magnet concentrator and Dynabeads (Invitrogen Co., Carlsbad, CA) strong anionic exchanger (SAX) were used to isolate lunasin from human plasma. Briefly, 40 μL of SAX was loaded in 1.5 mL microcentrifuge tubes placed in the magnet for 2 min. The supernatant was then discarded. The tube was removed from the magnet, and 100 μL of 50 mM sodium phosphate (pH 8.0) and 1 M NaCl were added to resuspend the beads. The mixture was placed in the magnet for 2 min and the supernatant discarded and treated with the same solution for a total of two washes. After preloading of SAX, it was equilibrated by being washed with 100 μL of 20 mM triethanolamine (pH 8.0) and 0.01 M NaCl (adsorption solution) for a total of two washes. After equilibration, triplicate 120 μL plasma samples (containing 60 μg of protein) from each of the five subjects were loaded in the beads, and the volume was adjusted to 200 μL with the adsorption solution. The mixture was left at room temperature for 2 min to allow the proteins to be adsorbed in the beads. The tube was then placed on the magnet for 2 min and the clear supernatant discarded. To ensure adsorption of proteins to SAX, the SAX/protein mixture was washed with adsorption solution three times. Proteins were desorbed from the SAX sequentially using different concentrations of NaCl ranging from 0.05 to 0.20 M in 20 mM

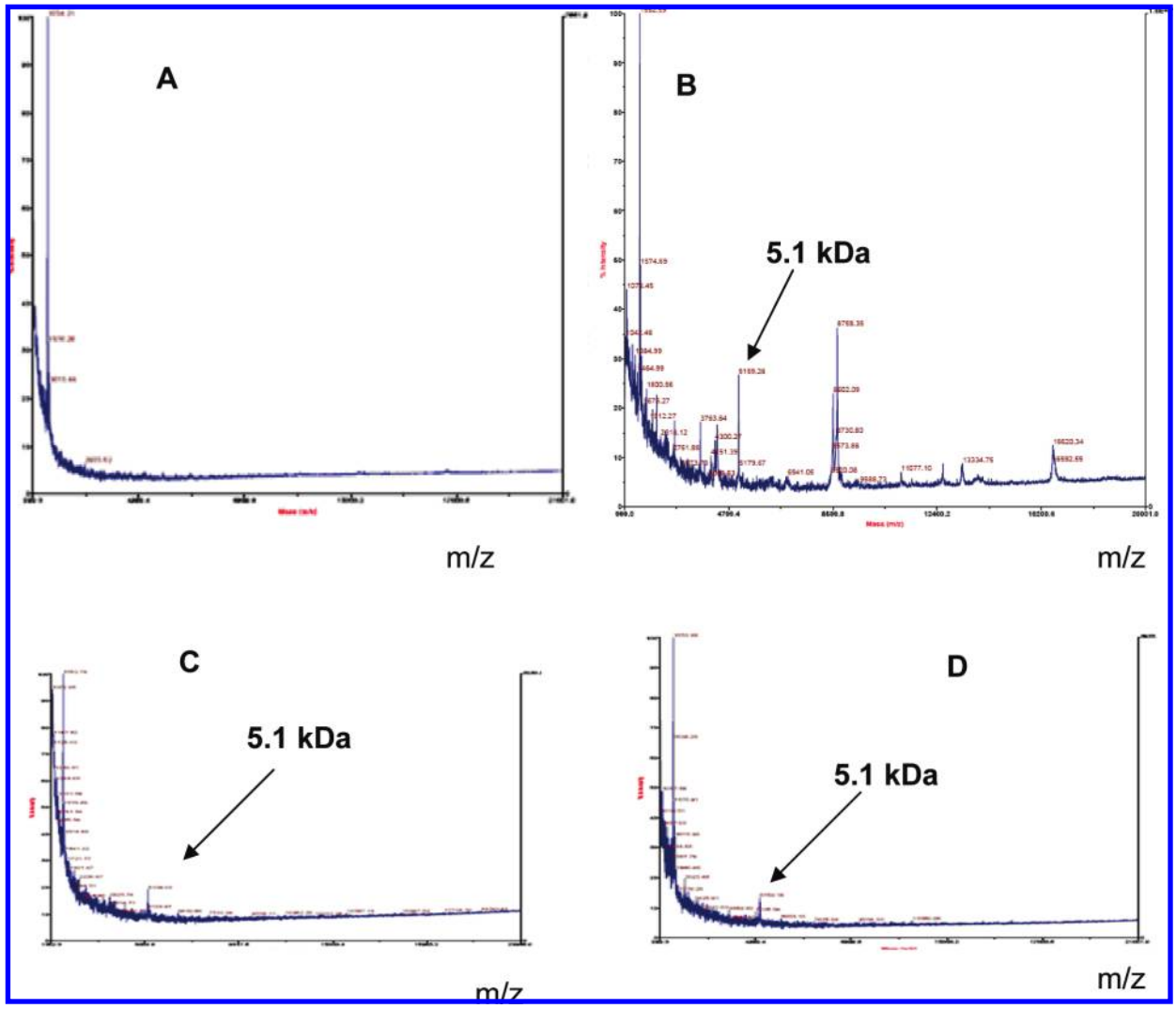

Figure 5. MALDI-TOF peptide mass mapping of plasma samples eluted from SAX beads with 20 mM triethanolamine and 0.2 M NaCl: (**A**) baseline plasma, (**B**) baseline plasma sample spiked with 1 μg of synthetic lunasin, (**C**) plasma sample taken 30 min after ingestion of 50 g of soy protein on day 5, and (**D**) plasma sample taken 1 h after ingestion of 50 g of soy protein on day 5.

triethanolamine (pH 8.0) (50 μL). The eluates (approximately 150 μL) from each of the triplicate plasma samples were pooled to have a sufficient volume of material to identify and quantify lunasin from each subject at each NaCl concentration. Baseline plasma and baseline plasma spiked with synthetic lunasin (5 and 10 μg) were used as controls.

Identification of Lunasin in Plasma Samples Spiked with Synthetic Lunasin. *Gel Electrophoresis.* Plasma samples (40 μL) spiked with 5 and 10 μg of synthetic lunasin were analyzed by sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE) using PhastGel Gradient 8-25 ready gels (GE Healthcare, Buckinghamshire, U.K.) in a Pharmacia LKB Phast System. Eluates (20 mM triethanolamine and 0.15 M NaCl) from SAX were diluted with tricine sample buffer (Bio-Rad Laboratories, Hercules, CA) with 2% β-mercaptoethanol and boiled for 4 min prior to being loaded. A prestained Kaleidoscope polypeptide standard (Bio-Rad Laboratories) was used. Two gels were run simultaneously (250 V, 10 mA). One was fixed with 10% acetic acid and 40% methanol for 30 min, stained with Bio-Safe Coomasie G250 (Bio-Rad Laboratories) for at least 1 h, and destained with 10% acetic acid for 30 min. The destained gel was washed with deionized water, and the gel picture was taken with a Kodak Image station 440 CF (Eastman Kodak Co., New Haven, CT). The other gel was transblotted (20 V, 25 mA) onto a polyvinylidene fluoride (PVDF) membrane for Western blot analysis.

Western Blot Analysis. The PVDF membrane with the transferred protein was blocked with 2% ECL Advance blocking agent (GE Healthcare) in Tris buffer saline with 1% Tween 20 (TBST) for 1 h, washed for 5 min three times with fresh changes of a 0.1% TBST solution (0.1% Tween 20 in Tris-buffered saline), and incubated with lunasin mouse monoclonal antibody (1:1000 dilution) for 16 h at 4 °C. After being washed, the membrane was incubated with ECL anti-mouse IgG−horseradish peroxidase conjugate (1:10000 dilution) for 3 h at room temperature. After being washed, the membrane was prepared for detection using chemiluminescent reagent (GE Healthcare) following the manufacturer's instructions. The membrane picture was taken with a Kodak Image station 440 CF (Eastman Kodak Co.).

Identification of Lunasin Using Matrix-Assisted Laser Desorption Ionization Time-of-Flight (MALDI-TOF) Peptide Mass Mapping. Molecular mass peptide mapping was conducted to identify lunasin in blood plasma (approximately 1 μL) at baseline and 30 min and 1 h after consumption of soy products. Samples were analyzed by MALDI-TOF using an Applied Biosystems (Foster City, CA) Voyager-DE STR at the protein facility of the University of Illinois. The following parameters were used in the analysis: linear mode of operation, positive polarity, and 3000−20000 Da scanning range. The molecular mass of lunasin in human plasma samples was determined by comparing the molecular mass MALDI-TOF profile against the profiles of baseline plasma, and baseline plasma spiked with 1 μg of synthetic lunasin.

Identification of Lunasin Using Liquid Chromatography−Tandem Mass Spectrometry (LC−MS/MS). Plasma eluates obtained after 20 mM triethanolamine (pH 8.0), 0.05 M NaCl washing were analyzed using LC−MS/MS to further confirm the presence of lunasin. For reduction, 25 μL of 10 mM DTT in 25 mM NH_4HCO_3 was added to the dried eluate and incubated at 56 °C with shaking for 1 h. The

Table 1. Peptides in the Tryptic Digest of Eluate from SAX Beads Loaded with Human Plasma

sample	monoisotopic mass	sequence[a]
baseline plasma[b]	833.49	INYIRR
	890.51	*GRINYIR*
	1046.61	*GRINYIRR*
	530.35	*QKKK*
	1806.95	*KKMEKELINLATMCR*
	602.31	*RNEGK*
	1426.57	FGPMIQCDLSSDD
	3204.63	SPKCQCKALQKIMENSEELEEKQKKK
plasma 1[c]	330.19	SPK
	890.50	*GRINYIR*
	1046.61	*GRINYIRR*
	530.35	*QKKK*
	602.31	*RNEGK*
	1806.95	*KKMEKELINLATMCR*
	656.33	**HIMEK**
	3476.75	FTILLISLLFCIAHTCSA**SKWQHQQDSCRK**
	2063.10	QKKKMEKELINATMCR
	446.21	NEGK
	472.27	**IQGR**
	1186.50	**WQHQQDSCR**
	860.45	**MEKIQGR**
plasma 2[d]	890.51	*GRINYIR*
	1046.61	*GRINYIRR*
	530.35	*QKKK*
	602.31	*RNEGK*
	1806.95	*KKMEKELINLATMCR*
	3476.75	FTILLISSLLFCIAHTCSA**SKWQHQQDSCRK**
	1456.77	**KQLQGVNLTPCEK**
	480.18	CQCK
	1382.60	CCTEMSELKSPK
	2063.11	QKKKMEKELINLATMCR

[a] Bold letters represent the amino acids that matched the 5 kDa lunasin soy peptide (SKWQHQQDSCRKQLQGVNLTPCEKHIMEKIQGRGDDDDDD DDD). Italic letters represent the amino acids that are common to all plasma samples. [b] Plasma sample taken at the start of the study, with no intake of soy protein for at least 1 week. [c] Plasma sample taken 30 min after ingestion of 50 g of soy protein on day 5. [d] Plasma sample taken 1 h after ingestion of 50 g of soy protein on day 5.

supernatant was removed, and 100 μL of 25 mM NH_4HCO_3 was added for rinsing. Alkylation was accomplished by adding 50 μL of 55 mM iodoacetamide in 25 mM NH_4HCO_3. The reaction was carried out in the dark at room temperature. The supernatant was removed and the sample rinsed with 100 μL of 25 mM NH_4HCO_3 followed by two 100 μL portions of 50% acetonitrile in 25 mM NH_4HCO_3. The sample was dried to completeness with vacuum. Trypsin digestion was accomplished by adding 25 μL of trypsin solution (12.5 ng of trypsin/μL of 25 mM NH_4HCO_3) carried out at 37 °C for 4−12 h. The digested peptides were extracted using 100 μL of 50% acetonitrile in 5% formic acid, and the sample mixture was sonicated for 10 min. The extraction was repeated twice, and the three extracts were pooled and dried. The dried peptides were dissolved in 10 μL of 5% acetonitrile in 0.1% formic acid for LC−MS/MS analysis. Liquid chromatography was carried out in digested peptides using a dC_{18} Atlantis nanoAcuity column [75 μm × 150 mm, 3 μm particle size (Waters, Milford, MA)] using 0.1% aqueous formic acid as solvent A and 50% acetonitrile with 0.1% formic acid as solvent B. A linear gradient from 1 to 90% B was run for 80 min and back to 1% B for 10 min with the flow rate maintained at 0.25 μL/min. Mass spectrometric analysis was carried out in a Q-Tof API-US nanoAcquity UPLC (Waters) tandem mass spectrometer equipped with an electron spray ion source. The Q-Tof instrument was operated in positive ion mode. The desolvation and source temperatures were set to 120 and 80 °C, respectively.

Quantification of Lunasin by an Enzyme-Linked Immunosorbent Assay (ELISA). Pooled eluates from triplicate plasma samples were analyzed for lunasin concentration by an ELISA following a previously reported protocol (*11, 15*). Briefly, 100 μL of eluate was plated on a 96-well plate and stored at 4 °C for at least 14 h. The plate was then washed with 0.01 M PBS with 0.05% Tween 20 (pH 7.4) using an ELX 50 Auto Strip Washer from Biotek Instruments (Winooski, VT). The plate was blocked by incubation with 300 μL of 5% BSA in TBS with 1% Tween 20 for 1 h, then washed, and incubated with 50 μL of lunasin mouse monoclonal antibody (1:4000 dilution) for 1 h at room temperature. After being incubated and washed, the plate was incubated with 50 μL of anti-mouse IgG−alkaline phosphatase conjugate secondary antibody (1:7000) for 1 h at room temperature. Color was developed by adding 100 μL of color reagent *p*-nitrophenyl phosphate to each well. The absorbance was read at 405 nm after incubation for 20 min using an ELISA plate reader ELX 808 IU from Biotek Instruments. The reaction was stopped by adding 100 μL of 3 N NaOH at 25 min and then read again at 35 min. The lunasin concentration was quantified against a standard curve of different concentrations of synthetic lunasin (**Figure 1**). Primary and secondary antibodies were diluted with 3% BSA, 1% Tween 20, and 0.05 M TBS buffer. All washings were conducted with 300 μL of washing solution, six times per well at the lowest dispensing rate (150 μL per well per second) and aspiration rate (5 mm/s) to avoid protein detachment.

Statistical Analysis. Data were analyzed by ANOVA using SAS version 9.1. Differences ($p < 0.05$) were considered statistically significant using Tukey mean separation.

RESULTS AND DISCUSSION

This is the first study which demonstrates that lunasin can be absorbed in humans, a key requirement for reaching target tissues and exerting its potential anticancer activity. Its presence and identity in blood after ingestion of soy protein were confirmed by various analytical methods. Validation of the isolation method for lunasin using SAX beads was conducted by spiking 5 and 10 μg of synthetic lunasin in 60 μL of the baseline plasma samples. **Figure 2** shows the gel electrophoresis profile (**A**) and Western blot analysis (**B**) of spiked baseline plasma samples eluted with 20 mM triethanolamine and 0.15 M NaCl. The plasma samples without synthetic lunasin (lane 1) and the one spiked with 5 μg of synthetic lunasin (lanes 2 and 4) did not show any positive immunoreactivity, in the latter case probably due to the low concentration. It is apparent from the Western blot figures that only the baseline sample spiked with 10 μg of synthetic lunasin (lanes 3 and 5) gave positive immunoreactivity with the lunasin monoclonal antibody. Even though there is a band in gel electrophoresis at ~5 kDa, this band did not interact with the lunasin monoclonal antibody, so it was not considered to be lunasin.

The effect of NaCl concentration on the amount of eluted lunasin from plasma samples is presented in **Figure 3**. It shows that most of the adsorbed lunasin (>80%) was eluted from the SAX beads with 0.15 and 0.20 M NaCl, though the concentration of lunasin was not statistically different ($p = 0.15$). This result is similar to the behavior of soybean lunasin reported earlier when lunasin was eluted from the diethylaminoethyl anion exchange column at 0.15−0.20 M NaCl (*11*).

Figure 4 shows the lunasin concentration in human plasma of subjects 30 min and 1 h after the intake of soy products on day 5. No lunasin was found in the baseline plasma samples. The lunasin concentration at 30 min ranged in the different subjects from 50.2 to 110.6 ng/mL of plasma (average ± standard deviation, 66.0 ± 25.4 ng/mL) and at 1 h from 33.5 to 122.7 ng/mL of plasma (average, 71.0 ± 32.8 ng/mL). The concentrations of lunasin found in plasma at 30 min and 1 h were not statistically different from each other ($p = 0.79$).

The lunasin concentration in the tested soy products was 0.16 ± 0.01 mg of lunasin/g of canned beans used to prepare the chili dish and 4.3 ± 0.4 mg of lunasin/g of soy protein powder used to prepare the soy milk shake. The portion size of chili was 300 g, and that of the soy milk shake was 28 g. On the basis of this, the total intake of lunasin from 50 g of protein from soy products was 155.5 mg/day. However, 97% of the lunasin is destroyed by gastrointestinal digestion (unpublished data using an in vitro model containing pepsin and pancreatin).

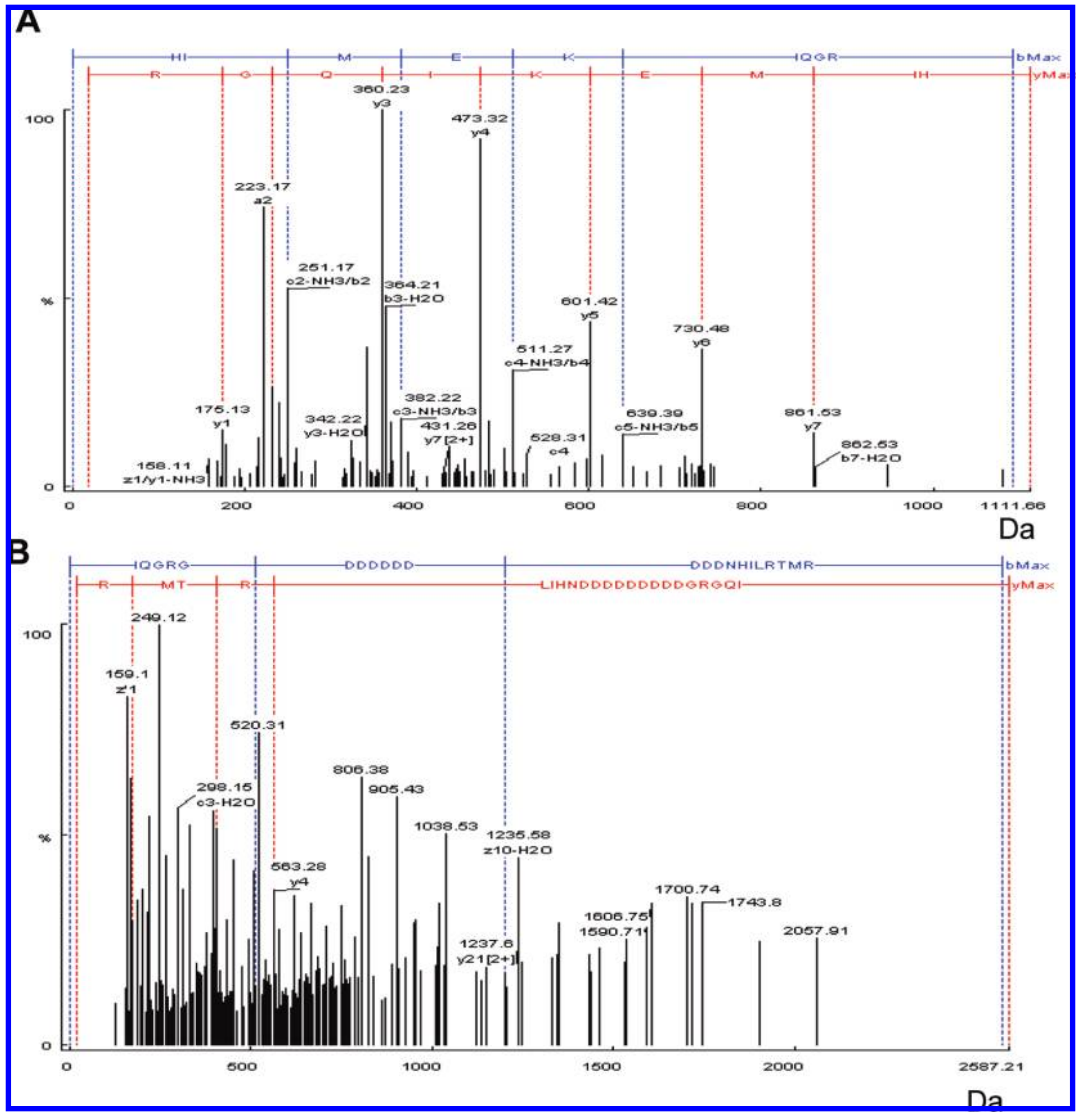

Figure 6. Mass spectra of the synthetic pure lunasin standard that matched the soybean lunasin sequence: (**A**) HIMEKIQGR and (**B**) IQGRGDDDDDDDDD.

Therefore, it can be assumed that only 4.7 mg of lunasin reached the absorption site in the intestine. In blood plasma, the average concentration of lunasin 1 h after ingestion was 71.0 ng/mL which is equivalent to 0.213 mg of lunasin in a reference person containing approximately 3 L of plasma. This amount represents an average of 4.5% lunasin absorption (range of 2.2−7.8%).

To validate the identification of lunasin from human plasma in this study, baseline plasma samples, baseline plasma spiked with 1 μg of synthetic lunasin, and plasma samples 30 min and 1 h after consumption of soy products were analyzed using MALDI-TOF. Panels **A** and **B** of **Figure 5** show the MALDI-TOF peptide mass mapping of the two baseline samples. Lunasin was not present in the baseline samples without spiking with lunasin (**Figure 5A**), while a 5.1 kDa peptide was found in the baseline plasma sample spiked with 1 μg of synthetic lunasin (**Figure 5B**). Panels **C** and **D** of **Figure 5** show the MALDI-TOF peptide mass mapping of 30 min and 1 h plasma samples from one of the five individuals. Results from each of the five individuals were similar. It is apparent from this figure that a 5 kDa peptide is present in the 30 min (**Figure 5C**) and 1 h (**Figure 5D**) plasma samples but not in the baseline plasma (**Figure 5A**). The fact that this MALDI-TOF lunasin profile is similar to the profile we reported earlier using synthetic lunasin (*11*) confirmed the presence of this compound in the blood of the human subjects.

LC−MS/MS analyses of synthetic lunasin and triethanolamine eluates from plasma samples at baseline and 30 min and 1 h after ingestion on day 5 were performed to further verify the identity and presence of lunasin in the plasma samples. **Table 1** lists the peptides found in the tryptic digests from these samples. Synthetic lunasin (100 μL of a 1 mg/mL solution with no plasma added) gave three peptides that corresponded to the lunasin sequence found in soybean, HIMEK, HIMEKIQGR, and IQGRGDDDDDDDDD. Analysis of the baseline plasma samples showed no generated peptides from trypsin digestion that corresponded to any sequence in the primary structure of lunasin. On the other hand, the plasma on day 5 of the feeding trial showed amino acid sequences corresponding to lunasin. The plasma sample concentrate taken 30 min after soy protein ingestion on day 5 showed the following amino acid sequences corresponding to lunasin: HIMEK, SKWQHQQDSCRK, IQGR, and MEKIQGR. The plasma sample concentrate taken at 1 h showed the following sequences that matched the primary structure of lunasin: SKWQHQQDSCRK and KQLQGVNLTPCEK. These lunasin concentrates reacted with the lunasin monoclonal antibody produced against the lunasin epitope,

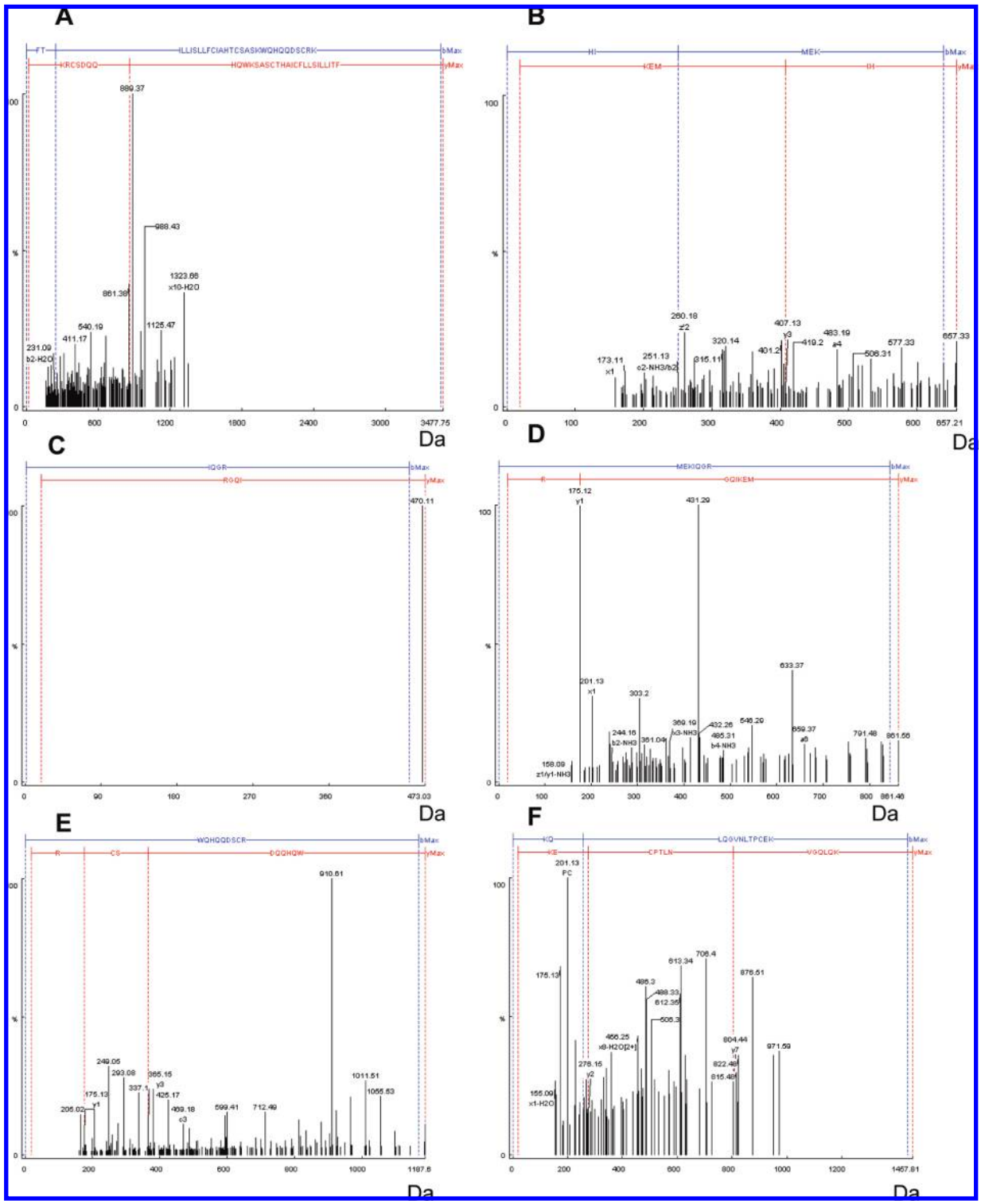

Figure 7. Mass spectra of peptides in the eluate from SAX beads loaded with human plasma withdrawn on day 5, 1 h after ingestion of 50 g of soy protein that matched the soybean lunasin sequence: (**A**) SKWQHQQDSCRK, (**B**) HIMEK, (**C**) IQGR, (**D**) MEKIQGR, (**E**) WQHQQDSCR, and (**F**) KQLQGVNLTPCEK.

EKHIMEKIQGRGDDDDD. **Figure 6** shows the spectra of only synthetic pure lunasin, while the spectra of peptides extracted, concentrated, and eluted from human plasma withdrawn 1 h after ingestion of 50 g of soy protein on day 5 are shown in **Figure 7**. These results indicate, as confirmed by various identification methods, that soybean lunasin is bioavailable in humans.

Lunasin has been isolated from soybean, barley, wheat, and other plant sources (*15*−*20*). Its chemopreventive properties are attributed to its ability to suppress colony formation induced by *ras*-oncogene and inhibition of core 3H-histone acetylation (*21*) and its ability to selectively kill cells that are being transformed or are newly transformed by disruption of the dynamic of histone acetylation and deacetylation (*16, 22, 23*). It inhibited pro-inflammatory responses in vitro such as nitric oxide and prostaglandin E_2 production and expression of inducible nitric oxide synthase and cyclooxygenase-2 at a concentration ranging from 10 to 300 μM (*11*). Also, it has

been shown that the formation of a colony transfected with an oncogene can be suppressed by soybean lunasin purified by ion-exchange chromatography and immunoaffinity chromatography at a concentration as low as 100 nM, while 10 μM lunasin was needed to inhibit 3H-histone acetylation (*21*). Another study showed that at 10 nM lunasin was able to inhibit acetylation of H3 histone by 20% while H4 histone acetylation was inhibited by 25% (*23*). These reported concentrations suggest that for lunasin to be bioactive in humans, large amounts of ingested soybean protein are required. However, long-term consumption may require smaller amounts.

The human bioavailability of Bowman-Birk inhibitor (BBI), another soy peptide, has also been reported previously (*24*, *25*). In a human study on the bioavailability of BBI, it was shown that BBI could be detected in the urine within 1−9 h of oral administration (*26*). In addition, the concentration of BBI metabolites recovered from urine of human subjects was estimated to be less than 0.02% of the BBI ingested (*27*).

Taken together, our results and those reported in the literature strongly suggest that bioactive peptides from soybean can be absorbed by the human gastrointestinal system. On the basis of our study, it can be concluded that lunasin can be found in circulation, a key biological requirement for its bioactivity when reaching target organs.

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