

Short Report

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Angiotensin-converting enzyme inhibitory effects of dairy- and soy-derived peptides in pre-hypertensive overweight men and women

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ABSTRACT:

Background: Hypertension is considered the most prevalent cardiovascular disorder and a significant public health problem. A functional food that could potentially impede progression into a hypertensive state in pre-hypertensive individuals is of significant interest to clinicians and consumers. *In vitro* and animal studies suggest the presence of potential ACE inhibitory dairy- and soy-derived peptides. Very few human-based research studies have been conducted to investigate the blood pressure lowering and/or ACE-inhibitory effects of whey and soy protein hydrolysates in humans. This pilot study tested the acute effects of 20g doses of whey and soy hydrolysates in pre-hypertensive, overweight men and postmenopausal women on serum ACE activity and blood pressure.

Findings: Using a randomized crossover design, four initial subjects received five treatments (unhydrolyzed casein, whey protein isolate, whey protein hydrolysate, soy protein isolate, soy protein hydrolysate) at different testing visits separated by three-day washout periods. Blood pressure and blood draws to measure ACE activity were taken at thirty minute intervals following treatment consumption. Both the soy protein and whey protein hydrolysates had notable *in vitro* ACE-inhibitory activity, both before and after heat treatment. No differences were observed among the protein treatments for either ACE activity or systolic blood pressure.

Conclusions: The results of this pilot study support a discrepancy between *in vitro* and human-based *in vivo* ACE-inhibitory acute effects of whey and soy protein hydrolysates, underscoring the need for further research to better understand potential explanations for these findings.

Key Words: ACE (Angiotensin-converting enzyme), Casein, Soy, Whey, Protein, Blood pressure, Dairy, Bioactive, Peptides

INTRODUCTION:

The renin-angiotensin-aldosterone system (RAAS) primarily increases blood pressure by producing the vasoconstrictor angiotensin II, facilitated by angiotensin-converting enzyme (ACE). Researchers have found antihypertensive potential in dairy-derived peptides [1-6]. The development of functional foods that may prevent hypertension in pre-hypertensive individuals is intriguing. We investigated the effects of an acute dose of whey- and soy-derived bioactive peptides on ACE activity and blood pressure in pre-hypertensive, overweight men and postmenopausal women.

METHODS:

Study Overview: This randomized, single-blind cross-over study compared whey protein hydrolysate (WPH), whey protein isolate (WPI), soy protein hydrolysate (SPH), soy protein isolate (SPI), and casein at 20g doses, an amount expected to elicit a biological effect based on a study reporting significant blood pressure reduction in humans consuming 20g WPH for six weeks [7]. To determine acute effects, measurements were taken up to 180 minutes post-treatment. See Figure 1 for study design.

← Controlled Diet →									
Pre-Testing Week 1	Visit 1	3Day Wash Out	Visit 2	3Day Wash Out	Visit 3	3Day Wash Out	Visit 4	3Day Wash Out	Visit 5
X	SPH*	X	WPI*	X	SPH*	X	Casein*	X	SPI*

Figure 1. Study Design: A Sample Schedule

*Each subject received the five treatments in a randomized order, each at a different visit: soy protein isolate (SPI), soy protein hydrolysate (SPH), whey protein isolate (WPI), whey protein hydrolysate

(WPH), casein. A controlled diet was followed throughout the study, beginning one week before testing; treatments were separated by 3-day washout periods. X: No treatment received

Subject Selection: Subjects were recruited through University of Minnesota Institutional Review Board (IRB)-approved campus fliers. The study protocol was reviewed and approved by the IRB and the Clinical and Translational Science Institute at the University of Minnesota. See Table 1 for recruitment eligibility.

Table 1. Study Eligibility

Body mass index (BMI)	25 – 34.9 kg/m ²
Age	≥ 40 years
SBP ^{*¥}	≥ 90 but ≤139 mm Hg
<i>and/or</i>	
DBP ^{†¥}	≤ 90 but > 80

Pertaining to females only:

No Menses for ≥ 12 months

No hormone replacement therapy medications

Had a complete hysterectomy ≥ 12 months prior to enrollment

* SBP: Systolic blood pressure

†DBP: Diastolic blood pressure

¥ Taken as the mean of 2 readings

Testing Visits: Subjects arrived to the DCRU by 0700 fasted from 1900 the day before and were instructed to lie supine with one pillow beginning 30 minutes before baseline, after which blood pressure assessment and blood draw for ACE activity were performed. Subjects consumed one of the five randomly assigned treatments, after which the Time 0 was marked. Blood sampling occurred at 30-minute intervals from Time 0 to 180 minutes, determined from a small preliminary study where nine subjects received 20g of one of the treatments and blood collection occurred at 15- and 30-minute intervals up to 300 minutes.

Controlled Diet: DCRU Nutrition Services designed a controlled diet to meet individual energy needs that was whey-, soy-, and dairy-free, providing 1g of sodium and 1.5g of potassium per 1,000 calories. All meals were provided and subjects were to report off-plan intake on food records and to return remains.

Production of hydrolysates:

Davisco Foods International, Inc. (Eden Prairie, MN) provided the WPI (BiPro) and WPH (Biozate 1). Archer Daniels Midland Company provided defatted soy flour samples (Decatur, IL). Minimally heat-treated and defatted soy flour was used to prepare SPI following methods

outlined by Tsumura et al [8]. Produced SPI was used to prepare SPH following selective hydrolysis with papain, mainly targeting hydrolysis of the β -conglycinin component [9].

Production of treatment cookies: Whey-derived peptides are typically delivered in beverages [3,7]. The insolubility of SPH in liquid and desire for optimal palatability influenced our decision to utilize the unique delivery form of a cookie. Twenty grams of the protein were divided into four cookies (Table 2) baked in a conventional oven at 449.7 K for seven minutes.

Table 2. Protein Treatment Ingredients

Ingredient	Amount (g)*
Protein**	24.83
Butter	21.59
Starch	21.59
Brown sugar	16.198
Splenda	1.94
Baking soda	0.539
Baking powder	0.269
Almond flavor	0.2159
Vanilla flavor	2.159
Walnut oil	2.159
Water	18.358
Chocolate chips	15.118
Total⁺ per cookie⁺⁺	125⁺ 28.94⁺⁺

* Amounts have been scaled up to account for losses during preparation

** Casein, soy protein hydrolysate, soy protein hydrolysate, whey protein isolate, or whey protein hydrolysate

⁺ Total weight of dough before dividing into 4 cookies

⁺⁺ Weight of each of the 4 cookies

***In vitro* ACE inhibition of hydrolysates before and after heating:** ACE inhibitory activity measurements of the hydrolysates were conducted, in triplicate, following the assay outlined by Otte et al [10] and Shalaby et al [11] using FA-PGG as substrate, with modifications adapted from Margatan et al [9]. The influence of thermal or pH instability on bioactivity or digestive survivability of peptides after baking is uncertain; therefore, SPH ACE inhibition was tested post thermal treatment similar to cookie baking conditions. *In vitro* inhibition was unaffected by heat treatment (data published elsewhere) [9].

Plasma ACE Activity Assay: At each time point, blood was collected (5mL), clotted, held at room temperature, and cold spun at 2091 RCF for 10 minutes. Serum (1mL) was aliquotted into cryogenic vials in duplicate, flash frozen with dry ice and alcohol, and stored at 210.8 K before shipment on dry ice to ARUP Laboratories (Salt Lake City, UT).

Statistical Analysis: Area under the curve (AUC) was calculated for each subject and treatment for ACE activity and systolic blood pressure (SBP) from baseline to 180 minutes ((Phoenix WinNonlin 6.3, Mountain View, CA, USA)). Because in vivo digestion of WPI, SPI, and casein could yield ACE-inhibitory peptides, differences across all treatments for both endpoints were evaluated using repeated measures ANOVA with post-hoc comparisons ((GraphPad InStat version 3.10 for Windows (GraphPad Software, San Diego California USA, www.graphpad.com))). Significance was set at $p < 0.05$.

RESULTS:

In vitro ACE inhibition of the SPH and WPH samples were similar, (IC_{50} of 0.177mg SPH protein/mL, 0.200mg WPH protein/mL [9] and fell within the reported range of IC_{50} values for ACE inhibitory peptides (0.046-0.930 mg protein/mL) [12-16]. No adverse events were reported, treatments were tolerated, and compliance was good. However, logistical challenges with study continuation encouraged an interim analysis of data from the first four subjects. A lack of significant differences between treatment proteins on either endpoint halted subject recruitment. Data from these four subjects are presented. See Table 3 for subject characteristics. Graphical representation of mean ACE activity (Figure 2) and SBP (Figure 3) over time, illustrate the variability among treatments.

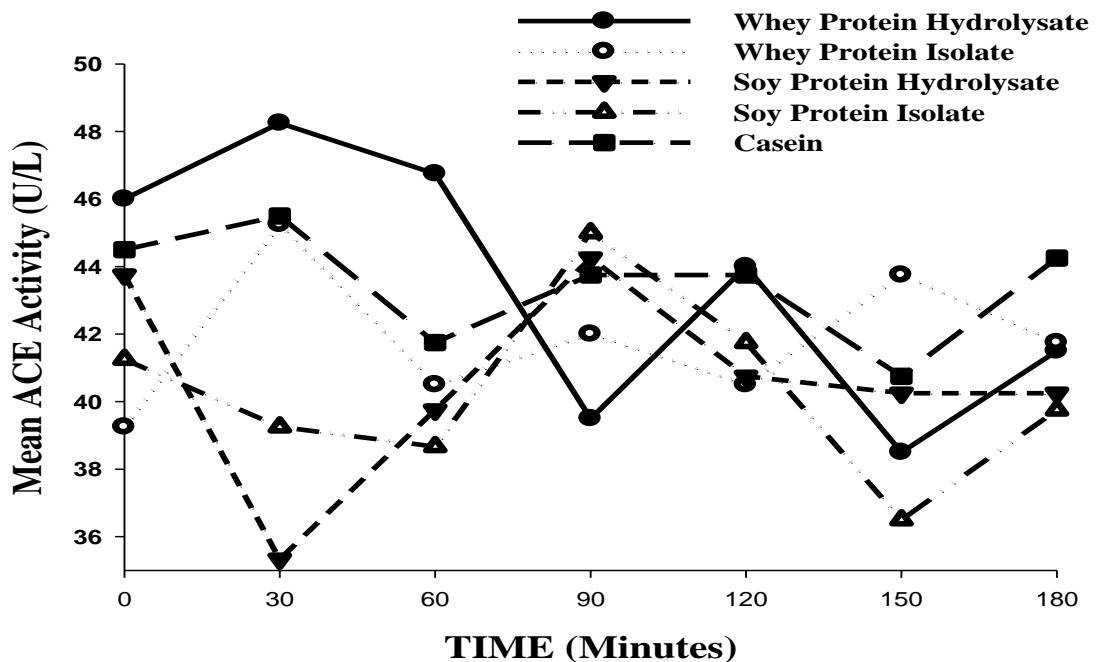


Figure 2. Mean ACE Activity vs Time by Treatment

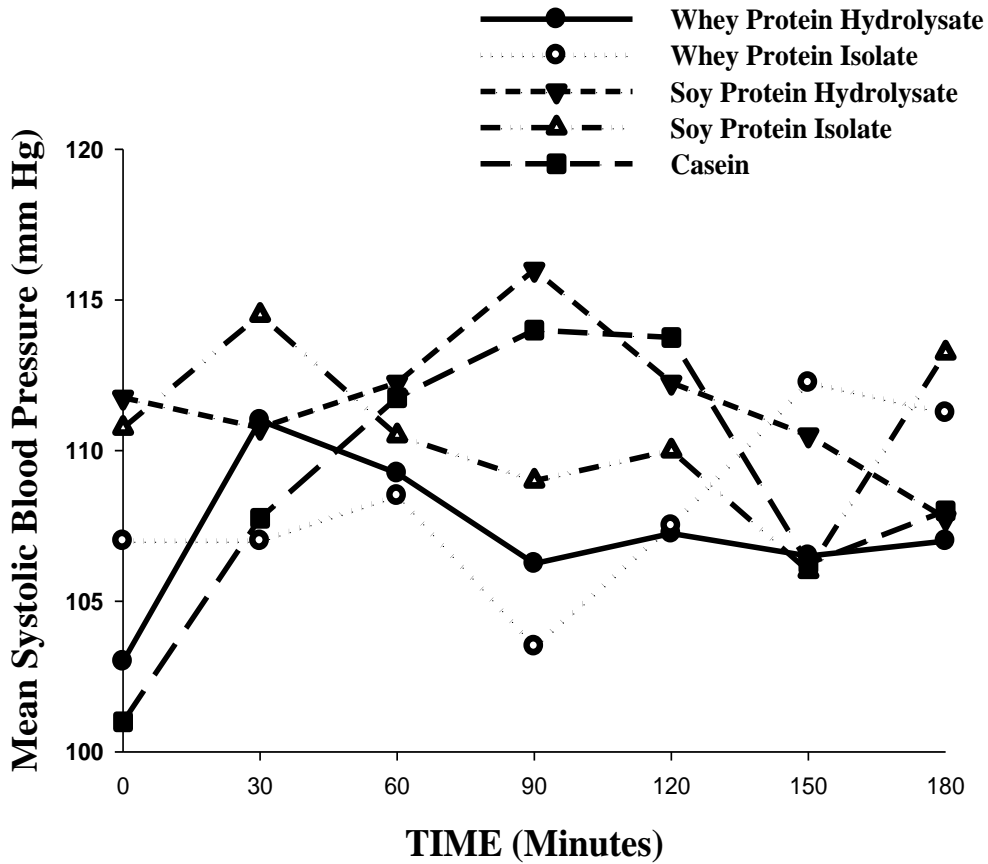


Figure 3. Mean Systolic Blood Pressure vs Time by Treatment

Table 3. Subject Characteristics

N	4 (2M, 2F)
Age*	58 ± 1.3 years
BMI*	28 ± 7 kg/m ²
SBP*	118 ± 7 mm Hg
DBP*†	81 ± 6 mm Hg

*Mean ± standard deviation

† Prehypertension may be defined by a DBP > 80 mmHg

Table 4. Angiotensin-Converting Enzyme (ACE) Area-Under-the-Curve (AUC) by Treatment

Treatment	Subject ID	AUC 60-180 min	AUC 0-180 min
Casein	85001	3825	6150
	85004	5445	7980
	85005	7110	10785
	85007	4170	6270
	Mean	5138	7796
	SD	1488	2161
SPI	85001	3405	4845
	85004	4845	7290
	85005	6892.5	10485
	85007	4575	6900
	Mean	4958	7429
	SD	1620	2272
SPH	85001	3405	4845
	85004	4845	7290
	85005	6892.5	10485
	85007	4680	7035
	Mean	4956	7414
	SD	1443	2323
WPI	85001	3885.00	5475.00
	85004	5490.00	8310.00
	85005	6630.00	10335.00
	85007	4080.00	6180.00
	Mean	5021.250	7575
	SD	1289	2200
WPH	85001	4410.00	6675.00
	85004	4545.00	7155.00
	85005	6930.00	10965.00
	85007	4050.00	6495.00
	Mean	4984	7823
	SD	1314	2113

Abbreviations: Soy protein isolate (SPI), soy protein hydrolysate (SPH), whey protein isolate (WPI), whey protein hydrolysate (WPH), casein

Table 5. Systolic Blood Pressure Area-Under-the-Curve (AUC) by Treatment

Treatment	Subject ID	AUC 60-180 min	AUC 0-180 min
Casein	85001	11280	16800
	85004	15360	22830
	85005	12045	18135
	85007	14580	21195
	Mean	13316	19740
	SD	1961	2762
SPI	85001	11190	16860
	85004	14790	22245
	85005	13125	20250
	85007	13320	20085
	Mean	13106	19860
	SD	1478	2228
SPH	85001	11625	17205
	85004	16140	24480
	85005	12810	19020
	85007	13275	19875
	Mean	13463	20145
	SD	1915	3097
WPI	85001	11055	16770
	85004	14715	22005
	85005	12885	19230
	85007	13320	19740
	Mean	12994	19436
	SD	1510	2148
WPH	85001	10890	16470
	85004	15495	23850
	85005	11730	17430
	85007	13260	19680
	Mean	12844	19358
	SD	2022	3283

Abbreviations: Soy protein isolate (SPI), soy protein hydrolysate (SPH), whey protein isolate (WPI), whey protein hydrolysate (WPH), casein

DISCUSSION:

Differences between treatments were undetected and no treatment lowered SBP or inhibited ACE to any observable degree. Strengths include use of a cross-over design, inclusion of *in vitro* ACE activity measurements, and a diet controlling potassium and sodium for consistency with close monitoring of compliance. However, only an acute dose of 20g was tested. This amount may be insufficient; studying the effects of multiple doses, or chronic dosing, over time would be more informative. Additionally, subjects were either mildly pre-hypertensive or normotensive; ACE-inhibitory potential may be more evident in pronounced hypertension. Other RAAS proteins were unevaluated to support or negate the proposition of ACE inhibition as the mechanism for reduced blood pressure. Finally, peptides were delivered in baked cookies. While heat stability in ACE-inhibitory activity was confirmed, stability to increased pH (potentially following added sodium bicarbonate and baking powder) is unknown. Although uncertain, pH and heat synergism and reactions occurring in heat conditions between proteins and reducing sugars, like the Maillard reaction, may influence bioactivity; these were not directly evaluated. Work is needed to determine optimal delivery to humans.

In conclusion, an acute dose of 20g of either SPH or WPH when delivered in a uniquely formulated cookie did not reduce SBP or ACE activity. Discrepancies between *in vitro* ACE inhibition by SPH and WPH and inadequate *in vivo* evidence warrant further research, particularly to determine peptide bioavailability for optimal dosing to achieve biological effects.

Competing Interests: The study was funded by Dairy Management, Inc., a company with an interest in whey proteins. Dairy Management, Inc. reviewed the study protocol as part of the grant application review process without any role in the collection, analysis, or interpretation of the data.

Authors' Contributions: MM was responsible for data collection, analysis, and interpretation, and drafted this manuscript. SS assisted with protocol development, data interpretation, and the editing of this manuscript. BI was responsible for the preparation of SPI and hydrolysates, measurement of *in vitro* ACE inhibition of WPH and SPH, formulation of the treatment cookies, and assisted with editing the manuscript. RB executed statistical analyses, worked with MM and CE in data interpretation and presentation, and assisted with editing this manuscript. CE was the principal investigator responsible for protocol development, and overall project management and supervision of data collection, analysis and interpretation. CE provided guidance and editing on the manuscript as senior author.

Authors' Information: MM is a Graduate Research Assistant; this project was conducted as part of her Master of Science in Nutrition. SS is an Associate Professor of Medicine at the University of MN. RB is a Professor in the Department of Experimental and Clinical Pharmacology at the University of MN. BI is an Assistant Professor of Food Science at the University of MN. CE is an Associate Professor of Nutrition at the University of MN.

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