The Chronic Effects of Whey Proteins on Blood Pressure, Vascular Function, and Inflammatory Markers in Overweight Individuals

ARTICLE *in* OBESITY · NOVEMBER 2009

Impact Factor: 4.39 · DOI: 10.1038/oby.2009.397 · Source: PubMed

2 AUTHORS, INCLUDING:

Sebely Pal
Collaboration for Evidence, Research and I…
65 PUBLICATIONS **1,079** CITATIONS

SEE PROFILE

The Chronic Effects of Whey Proteins on Blood Pressure, Vascular Function, and Inflammatory Markers in Overweight Individuals

Sebely Pal[1] and Vanessa Ellis[1]

Limited evidence suggests that dairy whey protein may be the major dairy component that is responsible for health benefits currently associated with increased dairy consumption. Whey proteins may reduce blood pressure and improve cardiovascular health. This study evaluated the effects of whey protein supplementation on blood pressure, vascular function and inflammatory markers compared to casein and glucose (control) supplementation in overweight/obese individuals. The subjects were randomized to either whey protein, casein or glucose supplementation for 12 weeks according to a parallel design. In all, 70 men and women with a mean (±s.e.m.) BMI (kg/m^2) of 31.3 ± 0.8 completed the study. Systolic blood pressure (SBP) decreased significantly at week 6 compared to baseline in the whey and casein groups, (P = 0.028 and P = 0.020, respectively) and at week 12 (P = 0.020, and P = 0.017, respectively). Diastolic blood pressure (DBP) decreased significantly compared to baseline in the whey and casein groups (P = 0.038 and P = 0.042, respectively) at week 12. DBP decreased significantly in the whey and casein groups (P = 0.025, P = 0.038, respectively) at week 12 compared to the control group. Augmentation index (AI) was significantly lower from baseline at 12 weeks (P = 0.021) in the whey group. AI decreased significantly in the whey group at 12 weeks compared to control (P = 0.006) and casein (P = 0.006). There were no significant changes in inflammatory markers within or between groups. This study demonstrated that supplementation with whey protein improves blood pressure and vascular function in overweight and obese individuals.

Obesity (2009) doi:10.1038/oby.2009.397

INTRODUCTION

The prevalence of the metabolic syndrome exceeds 20% among adults in many western countries (1). Metabolic syndrome significantly increases the risk of developing cardiovascular disease and type 2 diabetes mellitus. It consists of a combination of different metabolic abnormalities, such as abdominal obesity, atherogenic dyslipidemia, insulin resistance, glucose intolerance, impaired endothelial function, elevated blood pressure and inflammation (2).

Small population reductions in blood pressure of 2–5 mm Hg are translated into important reductions in mortality due to stroke and coronary heart disease and in total mortality (3). Although diet is acknowledged as a major determinant of blood pressure, the role of some foods has been investigated as preventive strategy. Epidemiological studies have shown an inverse relationship between the incidence of stroke and the consumption of milk and milk products. The Honolulu Heart Study reported that Japanese Americans who did not consume any milk had a twofold higher risk of stroke compared to persons consuming two or more 240-ml glasses of milk every day (4).

The renin–angiotensin system is an important regulator of blood pressure. Therefore, drugs that inhibit the renin–angiotensin system, either by inhibiting angiotensin-converting enzyme (ACE) or by blocking angiotensin receptors, are widely used in the treatment of hypertension. Milk proteins have been reported to inhibit ACE activity (5–9). There is also some *in vitro* evidence that whey proteins have an antihypertensive activity (6,7,10). A study by Kawase *et al.* (9) in humans showed that systolic blood pressure (SBP) was significantly lowered by the intake of fermented milk enriched in whey proteins after 8 weeks. However, a recent study by Lee *et al.* demonstrated that the daily consumption of 125 ml of a milk drink supplemented with whey peptides was not found to reduce blood pressure and/or inflammation markers (interleukin (IL)-6 and C-reactive protein (CRP)) in mildly hypertensive subjects (11). The mixed results of these limited studies examining the effects of whey proteins on blood pressure may be related to the low dose of whey protein used. The effect of whey proteins on vascular function and inflammation have not been examined previously. Therefore the aim of this study was to examine

[1]School of Public Health, Curtin Health Innovation Research Institute, ATN Centre for Metabolic Fitness, Curtin University of Technology, Perth, Western Australia. Correspondence: Sebely Pal (s.pal@curtin.edu.au)

Received 3 August 2009; accepted 6 October 2009; advance online publication 5 November 2009. doi:10.1038/oby.2009.397

the effect of whey protein isolate on blood pressure, vascular function and inflammatory markers compared to casein and glucose supplementation.

METHODS AND PROCEDURES

Subjects

Overweight and obese individuals between the ages of 18–65 with a BMI falling between 25–40 kg/m^2 were recruited from the local newspapers and TV in Perth, Australia. There were 380 responses of whom 97 were eligible to participate with 89 commencing the study. The screenings were initially conducted via telephone and online screening forms followed by screenings in person. Those who were suitable then attended a group orientation meeting where details of the study were explained before written consent was obtained. Exclusion criteria included those taking regular medications (such as lipid lowering, hypertensives) that were likely to affect the study outcomes, cancer within the last 5 years, smoking, pregnancy or lactation, major illnesses, eating disorders, over two standard alcoholic drinks per day, respiratory disease, type 1 or type 2 diabetes and cardiovascular events in the last 6 months. The study was approved by the Curtin University Human Research Ethics Committee (approval number HR 149/2007) and all subjects gave informed consent.

Study design and methodology

This was a randomized, single blind, parallel design study over a 12-week period with a 4-week washout period before commencement. The subjects were blinded in regard to the type of supplement received. They had no knowledge of the supplement type as they were all closely matched for taste and appearance. The silver packaging of the glucose, casein and whey protein supplements were visually identical. The supplement sachets were numerically coded which only the researcher distributing the supplements could decode. The subjects receiving the treatments and the investigators assessing the outcomes and analyzing the data, were blinded to the supplement assignment. Subjects were randomized to one of three groups, whey protein group, casein protein group, or the control group (glucose). After randomization, subjects were asked to consume one of the following coded supplement packages mixed with 250 ml water twice a day for 12 weeks: whey protein isolate, sodium caseinate (both containing 27 g protein) or glucose control (27 g glucose) (MG Nutritionals, Victoria, Australia). All supplements had equal kilojoule content at 525 kJ per sachet. The composition of the protein supplements are shown in Table 1. Subjects were instructed to take one sachet within 30 min before breakfast and one within 30 min before their evening meal. Subjects were asked to record their consumption of the sachets by marking tailored calendar tick boxes as well as keeping empty sachets to monitor compliance.

All subjects were asked to maintain their usual dietary intake and physical activity levels for the duration of the study. In order to maintain isocaloric diets due to the addition of a total of 1,050 kJ from two sachets of supplements per day, the subjects were given individual instruction from the study dietitian regarding dietary adherence to reduce their usual dietary intake by around 1,050 kJ per day to avoid the gaining of weight during the study period. This was achieved by way of guidelines listing exchanges in kJ for one sachet of supplement. This was monitored via food records which were completed every 2 weeks of the study (week 2, 4, 6, 8, and 12), on 2-week days and one weekend day to assess compliance. At the bottom of each diary sheet, subjects were asked to indicate how they adjusted their diet to compensate for the extra 1,050 kJ consumed per day. This in conjunction with analysis of the food records on FoodWorks 2007, enabled the study dietitian to determine if subjects were under or over-compensating and to contact the subject as appropriate. All other aspects of their dietary intake, such as macronutrient content, were to remain unchanged, apart from the consumption of the supplements. However, all subjects were instructed by the study dietitian to limit alcohol to two or less standard drinks for men and one or less standard drinks for women for the duration of the study. The subjects were also instructed to keep dairy intake to a minimum, at around one serve per day, for 4 weeks leading up to, and during, the study period. They were provided with instructions as to what constitutes a serve of dairy. Prior to commencement of the study, subjects completed a 7 day weighed food diary to ascertain their dairy intake. They were also asked to not take any supplements such as multivitamins, herbal or other, during this period. Any subjects who did not comply with these instructions did not commence the study. Energy and nutrient composition were calculated using FoodWorks 2007 (Xyris Software, Highgate Hill, Australia) based on the Australian food composition tables. Along with the biweekly food diaries, subjects were asked to complete the IPAC (Physical Activity questionnaires) to monitor physical activity levels to ensure that these remained constant throughout the duration of the study (12).

Assessments

Subjects were asked to visit Curtin University for measurements, in a fasted state and wearing light clothing, on three occasions, one for baseline measures, at 6 weeks and at 12 weeks. Body weight (UM-018 Digital Scales, Tanita, Tokyo, Japan) was recorded in light clothing. Height was measured to the nearest 0.1 cm using a stadiometer (26 SM 200 cm SECA, Hamburg, Germany) without shoes. Waist circumference was measured in the standing position at the narrowest area between the lateral lower rib and the iliac crest. Hip measurement was taken at the largest circumference of the lower abdomen. The waist and hip measurements were taken three times at baseline, week 6 and week 12. The average at each time point was then reported.

Blood pressure was measured with an automated, calibrated sphygmomanometer (Dinamap, Compact T, Critikon, Germany) with subjects in a supine position after resting for at least 10 min. The measurements were taken on the same arm three times at 1-min intervals. These readings were then averaged.

Table 1 Protein supplement nutritional information and typical composition in two sachets per day (60 g)

Nutritional information (g)	Whey protein isolate	Sodium caseinate
Protein (TN × 6.38)	54.0	54.3
Fat	0.30	0.72
Carbohydrate—lactose	0.30	0.12
Sodium	0.42	0.78
Calcium	0.09	0.06
Phosphorus	0.18	0.46
Typical composition (%)		
Protein	90.0	90.5
Branched chain amino acid profile % wt/wt		
Isoleucine	*6.8*	*4.8*
Leucine	*9.5*	*8.2*
Valine	*5.8*	*6.0*
Lactose	0.5	0.2
Fat	0.5	1.2
Moisture	2.2	1.3
Ash	3.7	3.7
Sweetener (sucralose)	0.06	0.06
Flavouring	3.0	3.0

Measurement of the augmentation index

Vascular measurements were performed by using the noninvasive SphygmoCor system (AtCor Medical, Sydney, Australia) which produces a pulse wave analysis based on measuring aortic root pressure waveform via a pen-like device on the peripheral pulse wave. All measurements were performed by the same operator. These measurements were taken three times with a quality index of at least 90% or over and the readings were averaged.

Measurement of plasma inflammatory markers

An 8.5 ml fasting blood sample was taken via venipuncture from subjects in the fasting state to measure circulating CRP, IL-6, and tumor necrosis factor-α (TNF-α). Serum was isolated by centrifugation at 3,000 rpm at 4 °C for 10 min °C and stored at −80 °C until the end of the study. Plasma IL-6, TNF-α and CRP levels were measured by a solid phase enzyme amplified sensitivity immunoassay performed on microtitre plates according to the manufacturer's instructions (TNF-α ELISA and IL-6 ELISA kits, BioSource, Belgium; CRP kits, Alpha Diagnostics International, San Antonio, Texas).

Statistical analysis

All subjects who completed the study were included in the data analysis. Statistical analysis was conducted using SPSS 17 for Windows (SPSS, Chicago, IL). Data are expressed as mean (s.e.m.) and assessed for normality. Comparison of baseline characteristics between each group was undertaken by one-way analysis of variance. Differences within groups were determined using a two-sided paired *t*-test. Using one-way analysis of covariance with the baseline data as the covariate, differences between groups at week 6 were conducted to assess immediate effects of intervention and at week 12 to assess longer term effects of supplementation. Statistical differences were analyzed further by *post hoc* analysis using the least square differences method. Percentage change between groups was calculated based on raw values of group means. Statistical significance was considered at $P < 0.05$.

This clinical trial has been registered with the Australian New Zealand Clinical Trials Registry. The registration number is: ACTRN12609000175279 and trial Web site: http://www.ANZCTR.org.au/ACTRN12609000175279.aspx.

RESULTS

Subjects

In all, 89 individuals were randomly assigned to either control, casein, or whey protein supplements for 12 weeks. Nineteen subjects withdrew from the study before baseline or within 4 weeks of baseline due to noncompliance for various reasons; five due to illness, three due to travel, eight due to personal reasons unrelated to diet and three lost to follow-up (five in the control, nine in the casein, and five in the whey group). Seventy subjects completed the 12-week study (control group: $n = 25$, 22 women, and 3 men; casein group: $n = 20$, 17 women and 3 men; whey protein group: $n = 25$, 21 women and 4 men). Subject characteristics in the three treatment arms at baseline were not significantly different (**Table 2**).

Diet and physical activity analysis

The self-reported composition of the study diets consumed during the 3-month study period is presented in **Table 3**. There were no significant differences in total energy, total fat, saturated fat, monounsaturated fat, polyunsaturated fat between control, casein, and whey protein groups. The contribution of carbohydrate to total energy intake was significantly lower in the casein group (36.2 ± 1.3, $P < 0.001$) and whey group (35.7 ± 1.1, $P < 0.001$) compared to control group (50.0 ± 1.0). The contribution of protein to total energy intake energy was significantly higher in subjects in the casein group (33.1 ± 0.9, $P < 0.001$) and whey group (31.5 ± 0.8, $P < 0.001$) compared to control group (16.1 ± 0.7). There were no significant changes in energy expenditure as recorded in 3-day physical activity logs between the different diet groups (data not shown).

Table 2 Subject characteristics at baseline

Characteristic	Control ($n = 25$)	Casein ($n = 20$)	Whey ($n = 25$)
Age (years)	48.4 ± 1.5	48.0 ± 2.1	48.5 ± 2.0
Weight (kg)	84.1 ± 1.8	82.9 ± 3.1	90.5 ± 3.4
BMI (kg/m^2)	30.6 ± 0.8	31.3 ± 0.9	32.0 ± 0.8
Body fat % (DXA)	35.4 ± 1.1	35.1 ± 2.1	37.6 ± 1.9
Waist circumference (cm)	93.7 ± 1.5	92.1 ± 2.1	95.9 ± 1.7
Waist: hip ratio	0.83 ± 0.01	0.81 ± 0.02	0.82 ± 0.01
Systolic blood pressure (mm Hg)	114.8 ± 2.26	118.1 ± 4.0	119.3 ± 3.2
Diastolic blood pressure (mm Hg)	66.0 ± 1.5	66.8 ± 2.2	64.1 ± 1.9
Augmentation index (%)	27.3 ± 2.1	27.6 ± 2.3	26.1 ± 2.3

Data are means ± s.e.m. ($n = 70$). There were no differences between the groups at baseline.

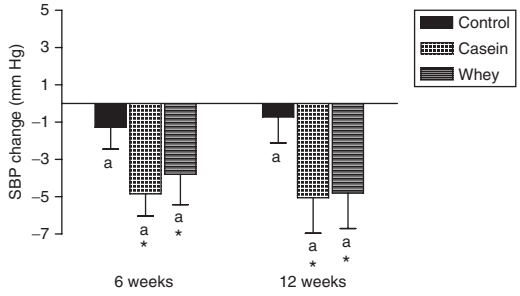

Figure 1 Changes in systolic blood pressure. The change in systolic blood pressure (SBP) from baseline to 6 weeks and 12 weeks following consumption of control, casein, or whey protein supplement. Data are mean ± s.e.m. Statistically significant differences from baseline are indicated by $^*P < 0.05$. Different letters above bar graphs indicate significance between groups at $P < 0.05$.

Blood pressure, vascular function, and inflammatory markers

Resting blood pressure was measured in the fasting state at baseline and at weeks 6 and 12. SBP decreased significantly by 4.2% at week 6 (113.2 ± 2.9 mm Hg; $P = 0.020$) and by 4.2% at week 12 (113.1 ± 3.02 mm Hg; $P = 0.017$) compared to baseline (118.1 ± 4.0 mm Hg), in the casein protein group (**Figure 1**). SBP decreased significantly by 3% at week 6 (115.5 ± 2.6 mm Hg; $P = 0.028$) and by 4% week 12 (114.5 ± 3.1 mm Hg; $P = 0.020$) compared to baseline (119.3 ± 3.2 mm Hg), in the whey protein group. There was no significant difference in SBP between diets at 12 weeks.

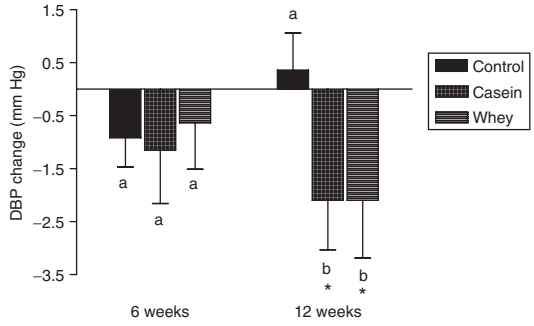

Figure 2 Changes in diastolic blood pressure. The change in diastolic blood pressure from baseline to 6 weeks and 12 weeks following consumption of control, casein, or whey protein supplement. Data are mean ± s.e.m. Statistically significant differences from baseline are indicated by *$P < 0.05$. Different letters above bar graphs indicate significance between groups at $P < 0.05$.

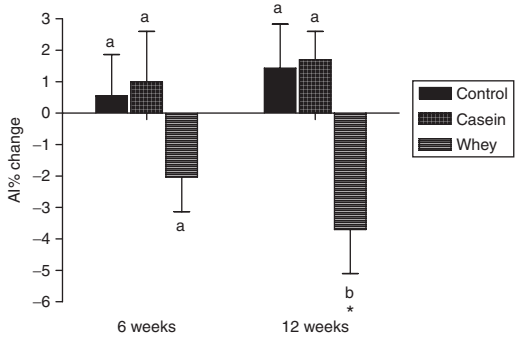

Figure 3 Changes in augmentation index (AI). The change in augmentation index from baseline to 6 weeks and 12 weeks following consumption of control, casein, or whey protein supplement. Data are mean ± s.e.m. Statistically significant differences from baseline are indicated by *$P < 0.05$. Different letters above bar graphs indicate significance between groups at $P < 0.05$.

There was a significant decrease of 3.3% in diastolic blood pressure (DBP) at week 12 (62.0 ± 1.7 mm Hg; $P = 0.038$) compared with baseline (64.1 ± 1.9 mm Hg) in the whey protein group (Figure 2). There was a significant decrease of 3.0% in DBP at week 12 (64.8 ± 2.00 mm Hg; $P = 0.042$) compared with baseline (66.8 ± 2.2 mm Hg) in the casein group. Compared to the control group, DBP was decreased significantly in the whey protein group ($P = 0.025$), and the casein group ($P = 0.038$) at week 12. There was no significant difference in DBP between the casein and whey protein groups ($P = 0.68$) at 12 weeks.

Measures of the central augmentation index (AI) were decreased by 7.3% from baseline at 6 weeks ($P = 0.055$) in the whey protein group. AI was significantly decreased by 14% from baseline at 12 weeks ($P = 0.021$) in the whey protein group (Figure 3). AI remained unchanged in the casein group at 6 and 12 weeks from baseline. AI was decreased significantly in the whey protein group at week 12 compared to control ($P = 0.006$) and casein groups ($P = 0.006$).

Table 4 shows the mean fasting levels of inflammatory markers from each intervention group at the three visits. There were no significant changes in IL-6, CRP, and TNF-α with time (baseline vs. 12 weeks) or between groups at the 12 week visit.

Table 3 Changes in nutritional variables and energy expenditure

	Baseline	Week 6	Week 12
Total energy intake (EI)			
Control	7535.9 ± 373.9	7385.0 ± 1197.8	7244.9 ± 337.9
Casein	7072.6 ± 212.4	6564.7 ± 263.3	6721.9 ± 235.8
Whey	7770.4 ± 415.5	7353.2 ± 447.0	7507.0 ± 385.1
Carbohydrate % of EI			
Control	45.7 ± 1.3	50.5 ± 0.9*a	51.5 ± 1.1#a
Casein	41.6 ± 1.5	37.2 ± 1.2*b	35.3 ± 1.3#b
Whey	43.7 ± 1.1	36.5 ± 0.9*b	34.9 ± 1.3#b
Protein intake % of EI			
Control	18.3 ± 0.7	16.4 ± 0.6*a	15.8 ± 0.7#a
Casein	20.5 ± 0.8	33.4 ± 1.1*b	32.9 ± 0.8#b
Whey	19.9 ± 0.8	31.3 ± 0.9*b	31.9 ± 0.8#b
Fat intake % of EI			
Control	33.0 ± 1.0	31.0 ± 0.9	30.1 ± 0.9
Casein	34.9 ± 1.1	30.4 ± 1.1	29.3 ± 1.0
Whey	33.7 ± 1.1	31.4 ± 1.0	29.7 ± 0.9
SFA % of total fat			
Control	42.0 ± 2.1	38.6 ± 1.2	41.4 ± 1.0
Casein	38.2 ± 1.8	41.0 ± 1.5	40.0 ± 1.0
Whey	42.4 ± 1.3	41.1 ± 1.3	41.3 ± 1.1
MUFA % of total fat			
Control	41.1 ± 0.7	41.2 ± 0.8	40.0 ± 0.5
Casein	42.0 ± 1.1	40.3 ± 1.0	41.6 ± 0.9
Whey	40.0 ± 0.8	41.3 ± 1.0	41.3 ± 0.8
PUFA % of total fat			
Control	16.9 ± 0.8	18.5 ± 0.9	18.5 ± 1.1
Casein	19.1 ± 1.6	18.4 ± 1.3	18.3 ± 0.8
Whey	16.4 ± 0.8	17.6 ± 0.7	16.8 ± 0.8

Values are daily mean ± s.e.m. ($n = 70$) of the nutritional data recorded in 3-day food diaries. Significant difference ($P < 0.05$) between group at 6 and 12 weeks is indicated by different letters.
*Significant difference ($P < 0.05$) within group (time 0 vs. time 6 weeks). #Significant difference ($P < 0.05$) within group (time 0 vs. time 12 weeks).

DISCUSSION

Supplementation with whey and casein protein for 12 weeks decreased SBP compared to baseline levels. Whey protein and casein supplementation also decreased DBP compared with control group at 12 weeks. However, there was no significant difference in SBP or DBP between whey and casein groups at 12 weeks. AI was decreased in the whey protein group at 12 weeks compared with baseline. AI was also significantly decreased at 12 weeks compared with control and casein group. Casein or whey supplementation had no effect on inflammatory markers. Overall, this study demonstrated that whey supplementation can significantly improve blood pressure and vascular function in overweight and obese individuals.

Table 4 Concentration of inflammatory markers

	Baseline	Week 12	Change
CRP (μg/ml)			
Control	3.17 ± 0.49	3.28 ± 0.57	0.105 ± 0.04
Casein	3.96 ± 0.59	3.88 ± 0.77	−0.080 ± 0.07
Whey	4.40 ± 0.61	3.75 ± 0.57	−0.653 ± 0.04
IL-6 (pg/ml)			
Control	14.26 ± 5.99	12.23 ± 4.79	−2.01 ± 1.38
Casein	17.62 ± 4.01	14.72 ± 6.11	−2.90 ± 1.21
Whey	18.68 ± 8.85	15.79 ± 7.28	−2.89 ± 1.78
TNF (μg/ml)			
Control	11.77 ± 1.19	9.80 ± 1.10	−1.96 ± 0.69
Casein	12.53 ± 1.64	11.41 ± 1.70	−1.11 ± 0.84
Whey	13.34 ± 1.37	11.03 ± 1.35	−2.31 ± 0.63

All values are mean ± s.e.m. Significance differences are defined as $P < 0.05$. There were no significant differences at baseline between diets. There were no significant differences within groups or between groups. Changes are defined as 12-week value minus baseline value.

ACE is a key enzyme in the regulation of peripheral blood pressure. Previous studies have demonstrated that milk proteins contain peptides that inhibit ACE activity (13,14). Casein-derived inhibitors of ACE are known as casokinins whereas whey-derived inhibitors are known as lactokinins (15). Several studies in spontaneously hypertensive rats show that these casokinins and lactokinins can significantly reduce blood pressure. SBP decreases ranging from 2–34 mm Hg have been reported (16,17). A limited number of clinical studies have been conducted examining the hypotensive effects of different milk proteins, with most examining the effects of casokinins. Sekiya *et al.* (18) were the first to demonstrate that consumption of 20 g/day of casein could reduce both DBP and SBP in hypertensive human volunteers. A number of other studies have shown similar effects of casein and fermented milk on SBP and DBP (8,19). Although for casokinins many *in vivo* studies in rats and in humans exist, there are only two human studies available for lactokinins, both with conflicting results. Consistent with the findings of this current study, the findings of Kawase *et al.* (9) showed that SBP was significantly lowered by the intake of fermented milk enriched with whey proteins after 8 weeks. In contrast, a recent study by Lee *et al.* (11) found the daily consumption of 125 ml of a milk drink supplemented with whey peptides (2.6 g) was not found to reduce blood pressure and/or inflammation markers (IL-6) in mildly hypertensive subjects compared to a lactose control drink (11). Previous *in vitro* tests of the whey peptides used in the milk drinks of this clinical study had demonstrated an ACE-inhibitory activity *in vitro*. The authors suggest that the lack of an effect *in vivo* could be related to the degradation of the peptides by intestinal or plasma peptidases before they could exert an effect on blood pressure. Another explanation could be an insufficient reabsorption of the peptides in the milk drink although proven to be bioactive *in vitro*. Also they suggest that a possible limitation of the study was that a higher dose of whey proteins may be required to exert a measurable biological effect in humans. In this study 54 g/day of whey protein was given to subjects which may explain the positive findings.

The American Heart Association (20) defines normal SBP and DBP as a reading <120 mm Hg and 80 mm Hg, respectively, prehypertensive as 120–139 mm Hg or 80–90 mm Hg, respectively and stage 1 hypertension as 140–159 mm Hg or 90–99 mm Hg, respectively. We had four subjects per group that fell into the prehypertensive category and only two subjects overall in the study that fell into the stage 1 hypertensive category. The blood pressure changes at 12 weeks in these individuals were similar to those individuals with normal blood pressure at baseline (data not shown).

Recent studies have shown that administration of ACE-inhibitors can have beneficial effects on inflammatory markers (21,22). Chronic inflammation is thought to play an important role in the pathogenesis of atherosclerosis (23,24). Therefore, inflammatory markers CRP, IL-6, TNF-α were investigated to examine whether dairy proteins had anti-inflammatory effects. Neither CRP, IL-6, nor TNF- α levels were affected by whey or casein supplementation. These findings are in agreement with a previous study examining the milk peptides on inflammation markers IL-6 and CRP (11).

Arterial stiffening is an independent risk factor for CVD (25,26). As people age, the central arteries gradually stiffen (27,28), with the rate of progression influenced by hypertension, diabetes, and atherosclerosis (29). The AI is an indicator of arterial stiffness and has been shown to be higher in those with hypercholesterolemia (30). It has been suggested that the increase in arterial stiffening may be associated with an increase in SBP and DBP (31–34). However, the mechanisms underlying age and disease-related arterial stiffening are not fully understood. Studies suggest that components of the renin–angiotensin system, matrix metalloproteinases, intracellular signaling, and extracellular matrix components may all be involved in this process (31–34). Given that whey proteins have been shown to reduce blood pressure (16–19) which may be associated with the angiotensin system, we hypothesize that these proteins may also have a beneficial effect on AI. There has been no previous study reporting the effects of dairy whey proteins on arterial stiffness. In this study, AI decreased significantly in the whey protein group by 21% compared with the control group and 23% compared to the casein at 12 weeks. There are many differences between whey and casein proteins which may be contributing to their differing in effect on AI. Whey protein, otherwise known as a "fast" protein due to its rapid emptying by the stomach, is delivered to the small intestine intact, enabling it to participate in a number of bioactivities in both the gut and circulation after it is secreted (35). In contrast, gastric emptying of casein is delayed due to its precipitation by gastric acids in the stomach and consequently its coagulation and is therefore referred to as a "slow" protein. As a result the postprandial appearance of plasma amino acids is much smaller than whey protein (35). Whey protein also consists of a heterogeneous group of proteins such as lactalbumin, β-lactoglobulin, immunoglobulin, lactoperoxidase and has a high content of branched chain amino

acids (leucine, isoleucine, and valine) which is thought to be responsible for its efficient metabolism after consumption (36). Further studies are required to elucidate whether these bioactive components or branched chain amino acids in whey may be responsible for the beneficial effect of AI in our study.

The physiologic effects of whey may be mediated by individual whey protein, peptide fractions or amino acids or by synergistic actions among them. It is unknown which components of whey proteins were responsible for the improvements in blood pressure and vascular function observed in this current study. Identifying the whey protein component(s) that may be responsible for such effects could form the basis for future studies. Future studies could also examine the effect of whey proteins on a hypertensive group.

In conclusion, this study demonstrated that supplementation with whey protein improves blood pressure and vascular function in overweight and obese individuals. Therefore, whey protein supplementation has the potential to be used as an added component in dietary plans and in functional foods aimed in the management of the metabolic syndrome risk factors.

ACKNOWLEDGMENTS

This study is a Dairy Australia initiative funded by the Dairy Service Levy and the Australian Government. Support for assays for inflammatory markers was provided by the ATN Centre for Metabolic Fitness. We thank Murray Goulburn Nutritionals (Victoria, Australia) for providing the supplements. The authors' responsibilities were as follows—VE coordinated the trial, data collection and input into the manuscript. SP conceived and designed the study, wrote the manuscript, supervised the study and the statistical analysis.

DISCLOSURE

The authors declared no conflict of interest.

REFERENCES

1. Balkau B, Vernay M, Mhamdi L *et al*.; D.E.S.I.R. Study Group. The incidence and persistence of the NCEP (National Cholesterol Education Program) metabolic syndrome. The French D.E.S.I.R. study. *Diabetes Metab* 2003;29:526–532.
2. Grundy SM, Brewer HB Jr, Cleeman JI, Smith SC Jr, Lenfant C, National Heart, Lung, and Blood Institute; American Heart Association. Definition of metabolic syndrome: report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. *Circulation* 2004;109:433–438.
3. Appel LJ, Brands MW, Daniels SR *et al*.; American Heart Association. Dietary approaches to prevent and treat hypertension: a scientific statement from the American Heart Association. *Hypertension* 2006;47:296–308.
4. Abbott RD, Curb JD, Rodriguez BL *et al*. Effect of dietary calcium and milk consumption on risk of thromboembolic stroke in older middle-aged men. The Honolulu Heart Program. *Stroke* 1996;27:813–818.
5. Yamamoto N, Takano T. Antihypertensive peptides derived from milk proteins. *Nahrung* 1999;43:159–164.
6. FitzGerald RJ, Meisel H. Lactokinins: whey protein-derived ACE inhibitory peptides. *Nahrung* 1999;43:165–167.
7. Mullaly M, Meisel H, FitzGerald R. Angiotensin-I-converting enzyme inhibitory activities of gastric and pancreatic proteinase digest of whey proteins. *Int Dairy* 1997;7:299–303.
8. Seppo L, Jauhiainen T, Poussa T, Korpela R. A fermented milk high in bioactive peptides has a blood pressure-lowering effect in hypertensive subjects. *Am J Clin Nutr* 2003;77:326–330.
9. Kawase M, Hashimoto H, Hosoda M, Morita H, Hosono A. Effect of administration of fermented milk containing whey protein concentrate to rats and healthy men on serum lipids and blood pressure. *J Dairy Sci* 2000;83:255–263.
10. Korhonen H, Pihlanto-Leppala A, Rantamaki P, Tupasela T. The functional and biological properties of whey proteins: prospects for the development of functional foods. *Agric Food Sci* 1998;7:283–296.
11. Lee YM, Skurk T, Hennig M, Hauner H. Effect of a milk drink supplemented with whey peptides on blood pressure in patients with mild hypertension. *Eur J Nutr* 2007;46:21–27.
12. Craig CL, Marshall AL, Sjöström M *et al*. International physical activity questionnaire: 12-country reliability and validity. *Med Sci Sports Exerc* 2003;35:1381–1395.
13. FitzGerald R, Meisel H. Lactokinins: whey protein derived ACE inhibitory peptides. *Nahrung* 1999;43:165–167.
14. Takano T. Milk derived peptides and hypertension reduction. *Int Dairy J* 1998;8:375–381.
15. FitzGerald RJ, Meisel H. Milk protein-derived peptide inhibitors of angiotensin-I-converting enzyme. *Br J Nutr* 2000;84(Suppl 1):S33–S37.
16. Abubakar A, Tadao S, Kitazawa H, Kawai Y, Itoh T. Structural analysis of new antihypertensive peptides derived from cheese whey protein by proteinase K digestion. *J Dairy Sci* 1998;81:3131–3138.
17. Nurminen ML, Sipola M, Kaarto H *et al*. Alpha-lactorphin lowers blood pressure measured by radiotelemetry in normotensive and spontaneously hypertensive rats. *Life Sci* 2000;66:1535–1543.
18. Sekiya S, Kobayashi Y, Kita E, Imamura Y, Toyama S. Antihypertensive effects of tryptic hydrolysate of casein on normotensive and hypertensive volunteers (in Japanese). *J. Jap. Soc. Nutr. Food Sci* 1992;45:513–517.
19. Hata Y, Yamamoto M, Ohni M *et al*. A placebo-controlled study of the effect of sour milk on blood pressure in hypertensive subjects. *Am J Clin Nutr* 1996;64:767–771.
20. Chobanian AV, Bakris GL, Black HR *et al*.; Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. National Heart, Lung, and Blood Institute. Seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. *Hypertension* 2003;42:1206–1252.
21. Prasad K. C-reactive protein (CRP)-lowering agents. *Cardiovasc Drug Rev* 2006;24:33–50.
22. Andrzejczak D, Górska D, Czarnecka E. Influence of enalapril, quinapril and losartan on lipopolysaccharide (LPS)-induced serum concentrations of TNF-alpha, IL-1 beta, IL-6 in spontaneously hypertensive rats (SHR). *Pharmacol Rep* 2007;59:437–446.
23. Humbert M, Monti G, Brenot F *et al*. Increased interleukin-1 and interleukin-6 serum concentrations in severe primary pulmonary hypertension. *Am J Respir Crit Care Med* 1995;151:1628–1631.
24. Matthews DR, Hosker JP, Rudenski AS *et al*. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985;28:412–419.
25. Casey DP, Pierce GL, Howe KS, Mering MC, Braith RW. Effect of resistance training on arterial wave reflection and brachial artery reactivity in normotensive postmenopausal women. *Eur J Appl Physiol* 2007;100:403–408.
26. Qureshi G, Brown R, Salciccioli L *et al*. Relationship between aortic atherosclerosis and non-invasive measures of arterial stiffness. *Atherosclerosis* 2007;195:e190–e194.
27. Greenwald SE. Ageing of the conduit arteries. *J Pathol* 2007;211:157–172.
28. McEniery CM, Wilkinson IB, Avolio AP. Age, hypertension and arterial function. *Clin Exp Pharmacol Physiol* 2007;34:665–671.
29. Benetos A, Waeber B, Izzo J *et al*. Influence of age, risk factors, and cardiovascular and renal disease on arterial stiffness: clinical applications. *Am J Hypertens* 2002;15:1101–1108.
30. Wilkinson IB, Prasad K, Hall IR *et al*. Increased central pulse pressure and augmentation index in subjects with hypercholesterolemia. *J Am Coll Cardiol* 2002;39:1005–1011.
31. Safar ME. Systolic blood pressure, pulse pressure and arterial stiffness as cardiovascular risk factors. *Curr Opin Nephrol Hypertens* 2001;10:257–261.
32. Blacher J, Safar ME. Large-artery stiffness, hypertension and cardiovascular risk in older patients. *Nat Clin Pract Cardiovasc Med* 2005;2:450–455.
33. Schiffrin EL. Remodeling of resistance arteries in essential hypertension and effects of antihypertensive treatment. *Am J Hypertens* 2004;17:1192–1200.
34. Tomiyama H, Hashimoto H, Hirayama Y *et al*. Synergistic acceleration of arterial stiffening in the presence of raised blood pressure and raised plasma glucose. *Hypertension* 2006;47:180–188.
35. Boirie Y, Dangin M, Gachon P *et al*. Slow and fast dietary proteins differently modulate postprandial protein accretion. *Proc Natl Acad Sci USA* 1997;94:14930–14935.
36. Bastian E, Harper WJ. Emerging health benefits of whey. *Dairy Council Digest* 2003;74:31–36.