Eur J Nutr (2007) 46:21–27
DOI 10.1007/s00394-006-0625-8

ORIGINAL CONTRIBUTION

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Effect of a milk drink supplemented with whey peptides on blood pressure in patients with mild hypertension

Received: 3 November 2005
Accepted: 27 October 2006
Published online: 15 December 2006

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Abstract *Background* Inhibition of the angiotensin I-converting enzyme (ACE) is a widely used principle for the treatment of hypertension. Fermentation of milk proteins was shown to lead to the formation of peptides with ACE-inhibiting activity. Milk products with ACE-inhibiting peptides may provide a useful approach to prevent or treat hypertension. *Aim of the study* To investigate the effect of a milk drink supplemented with whey peptides on blood pressure in mildly hypertensive subjects. *Methods* A randomized, placebo-controlled, double blind clinical trial in two parallel groups was performed. A total of 54 hypertensive patients received either 125 ml of a milk drink supplemented with whey peptides every morning or a control product for 12 weeks after a run-in period of 2 weeks. Previous in vitro tests of the whey powder demonstrated ACE-inhibitory activity. Blood pressure was measured at 0, 2, 4, 8, and 12 weeks. Fasting blood samples were collected at 0, 4, 8, and 12 weeks for analysis of metabolic and inflammatory variables. *Results* Resting systolic and diastolic blood pressure values did not change in the milk drink group 144.1 ± 8.6/91.0 ± 5.5 mmHg at baseline vs. 143.7 ± 13.5/90.4 ± 6.5 mmHg after 12 weeks. In the control group systolic ($p = 0.0431$) and diastolic ($p = 0.0081$) blood pressure was significantly reduced 140.6 ± 11.7/90.3 ± 5.8 mmHg at baseline vs. 137.0 ± 14.4/87.7 ± 6.6 mmHg after 12 weeks. There was no difference between the two groups at any time point. No changes were seen when the results of the 24-h continuous blood pressure monitoring were compared after 12 weeks. No significant changes were detected for circulating levels of selected inflammation markers (interleukin (IL)-6, C-reactive protein (CRP), number of leukocytes, and plasminogen activator inhibitor-1 (PAI-1) as well as for metabolic variables (insulin, plasma glucose, and lipids). *Conclusions* 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 in mildly hypertensive subjects, although preceding in vitro tests showed a potent ACE-inhibition.

Key words angiotensin I-converting enzyme – whey – bioactive peptides – hypertension

EJN 625

Introduction

Hypertension is a frequent condition in the European population with almost 55% of the 35–64-year-old affected. A recent study revealed that the majority of the affected persons are either not at all or insufficiently treated. Especially among patients with blood pressure values of ≥140/90 and <160/95 mmHg three quarters were without medical treatment [1]. This is a rather unsatisfactory situation as elevated blood pressure is a powerful risk factor for cardiovascular disease [2]. In Germany, approximately 180,000 people suffer a stroke every year mostly of ischemic origin. Stroke survivors are highly impaired in their cognitive and physical functions and may become an economic burden for the health care system. In view of this challenge there is growing interest in the role of nutrition and lifestyle for the prevention or early treatment of hypertension [2].

Epidemiological studies show an inverse relationship between the consumption of milk and milk products and the incidence of stroke. The Honolulu Heart Study reported that Americans of Japanese origin who did not consume any milk had a twofold higher risk of stroke in an observation period of 22 years compared to persons consuming two or more 240-ml-glasses of milk every day (7.9 vs. 3.9 cases per 100) [3].

More recent studies suggest a blood pressure lowering potential of milk products. Fermentation of milk proteins leads to the production of peptides that have angiotensin I-converting enzyme (ACE)-inhibiting activity [4]. ACE catalyzes the conversion of the decapeptide angiotensin I to the octapeptide angiotensin II which is acting as a vasoconstricting substance. ACE-inhibition has become an established principle in the treatment of hypertension in clinical medicine.

Oligopeptides derived from bovine milk were reported to inhibit ACE activity [4–16]. Recent clinical studies in humans demonstrated a blood pressure lowering effect of functional milk drinks. In a Japanese study, a milk product fermented with a starter containing *Lactobacillus helveticus* and *Saccharomyces cerevisiae* was tested in hypertensive patients and a significant decrease in systolic and diastolic blood pressure by 14 and 7 mmHg, respectively, was measured compared to placebo after 8 weeks of daily intake [5]. In a Finnish study, daily ingestion of 150 ml milk fermented with *Lb. helveticus LBK-16H* for 21 weeks resulted in a reduction of systolic and diastolic blood pressure by 7 and 4 mmHg, respectively, compared to the control group [6].

There is also evidence for an anti-hypertensive activity of whey-protein derived peptides from in vitro studies [7–9]. However, the in vitro findings have not been confirmed in human studies so far. The aim of this study was to examine the potential anti-hypertensive effect of a newly developed milk drink supplemented with whey peptides for which preceding in vitro tests demonstrated an ACE-inhibitory activity in a group of mildly hypertensive patients.

Subjects and methods

Subjects

The volunteers were recruited by advertisements in local newspapers between June and September 2004. One hundred and nineteen subjects entered the screening procedure. Fifty-four subjects met the eligibility criteria, were enrolled, and randomized. The primary inclusion criteria were stable mild hypertension defined as diastolic blood pressure >85 mmHg and <95 mmHg and/or a systolic blood pressure >135 mmHg and <160 mmHg, age between 30 and 65 years, and BMI <40 kg/m^2. Exclusion criteria were treatment with ACE-inhibitors or angiotensin II subtype 1 receptor blockers, severe intolerance for lactose, known diabetes mellitus, and a recent cardiovascular event.

Written informed consent was given by all subjects prior to the beginning of the study. The study protocol was approved by the ethical committee of the Technical University of Munich.

Study design

This was a randomized, double blind, placebo-controlled study in two parallel groups over a period of 12 weeks with a 2-week run-in period. The participants received a daily dose of 125 ml of either the test or placebo product in the morning. Blood pressure was measured in the morning after 0, 2, 4, 8, and 12 weeks by the same person and additional ambulatory continuous blood pressure monitoring was done at time points 0 and 12 weeks.

Blood samples were drawn after an overnight fast before the milk drinks were taken after 0, 4, 8, and 12 weeks. Compliance was monitored by counting the number of empty bottles, which the participants brought in. At each visit adverse effects, changes in body weight, medication, lifestyle, and acute illnesses were documented. All patients were asked to maintain their lifestyle and keep their body weight stable. A structured dietary intake questionnaire was used to assess dietary intake at the beginning and end of the intervention.

Table 1 Composition per 100 g of the milk drink containing whey peptides and the control drink

	Milk drink	Control drink
Total solids (g)	20.81	15.54
Fat (g)	0.12	0.02
Protein[a] (g)	2.60	0.20
Carbohydrates (g)	15.09	14.95
Calcium (mg)	397	<10

[a]Determined by Kjeldal's method.

Test and control products

The milk drinks were provided by Unternehmensgruppe Theo Müller GmbH & Co. KG (Aretsried, Germany). Skim milk was the basis for the drinks. Fruit concentrate, water, sweetener, and flavorings were added. Only the test drink contained whey powder. The control drink contained no whey powder but lactose and acidifier were added to be similar in taste. Both drinks were produced in an all-in-one-procedure and heated for 2 min at 95°C. The whey peptides were obtained from acid reduced mineral whey powder (ARMWP), which was achieved as a byproduct from sour milk cheese production, nano filtration and consecutive spray drying. The presumed bioactive peptides were in the whey powder and in vitro tests have been originally performed by N-zyme (Darmstadt, Germany) using a modification of the method by Hyun [18], which revealed a significant ACE-inhibiting effect.

Briefly, the ACE assay consisted of

- 225 µl 5 mM HHL in Na-borate buffer, pH 8.3; 0.4 M NaCl
- 50 µl 120 mU/ml ACE in borate buffer, pH 8.3; 0.4 M NaCl
- 25 µl sample/reference

10 minutes pre-incubation at 37°C, 30 min incubation at 37°C, and then stopping of the reaction with 20 µl 5 M HCl. For analysis, 200 µl were used of the liberated hippuric acid using RP-HPLC. The IC_{50} values were then determined by regression or graphically. Table 1 shows the composition of the test and the control milk drink.

Methods

Blood pressure was measured with a sphygmomanometer (Maxi Stabil 3; WelchAllyn, Jungingen, Germany) on the left arm in a sitting position after a 20-min rest. Four measurements were done at an interval of 2 min. The last three measurements were averaged and taken as the actual blood pressure value. The ambulatory continuous blood pressure monitoring for 24 h was performed with the Tonoport V system according to the instructions of the manufacturer (GE Medical System, Milwaukee, USA).

Blood samples for the assessment of fibrinolytic activity were immediately transported to the laboratory on ice, centrifuged at 4°C and stored at −20°C until assayed. The fibrinolytic function was assessed by measuring PAI-1 activity using Berichrom* PAI (Dade Behring, Marburg, Germany). Control sera were in the allowed range as given in the instructions of the manufacturer. IL-6 was assessed by a bead based assay (BioRad, Munich, Germany). The inter- and intra-assay coefficients of variation were <10%. CRP was measured by means of immunonephelometry using BNA II (Dade Behring, Marburg, Germany). Insulin was determined with Insulin RIA coated tubes (DPC-Biermann, Bad Nauheim, Germany). The homeostasis model assessment of insulin resistance (HOMA) was calculated according to the published formula: fasting glucose (mmol/l) × fasting insulin (µU/ml)/22.5 [19]. Other serum variables were analyzed by a certified laboratory for clinical studies (Labor Dr. Tiller & Kollegen, Munich, Germany).

Statistical methods

Based on a previous study [6], eight subjects per group were calculated to be sufficient to detect a 6 mmHg difference in systolic blood pressure between the milk drink and placebo group from baseline with a 90% power at a significance level of $\alpha = 0.05$, assuming a standard deviation (SD) of 4 mmHg.

Analysis was based on the intention-to-treat-principle (ITT-principle): Only randomized patients with at least one post-baseline measurement were considered for the efficacy analysis. Missing values were handled according to the last-value-carry-forward principle: the final available measurement was considered as last measurement under treatment.

For descriptive purposes, quantitative data is presented as mean, including standard deviation or 95%-confidence interval. For qualitative data we used frequencies (n) and percentages. To compare baseline measurements to study-end measurements, the Student's t-test for paired samples was used for each treatment group. The between-treatment-comparisons were performed by the t-test for unpaired samples. The Satterthwaite correction was used in case of unequal variances, assessed by the Levene-test. All statistical tests are two-sided at the 5% significance level. There was no adjustment for multiple tests.

All analyses were performed using SAS ©, version 9.1.

Table 2 Demographic characteristics of the ITT-population at baseline

	Milk drink group	Control group	*P*-values
Number of subjects	27	26	
Gender (%) (male/female)	51.9/48.1	61.5/38.5	0.583
Age (years)	55.3 ± 10.4	47.8 ± 11.6	0.014
RR[a] systolic (mmHg)	144.1 ± 8.6	140.6 ± 11.7	0.863
RR[a] diastolic (mmHg)	91.0 ± 5.5	90.3 ± 5.8	0.575
Body height (cm)	172.0 ± 8.3	174.0 ± 10.3	0.436
Body weight (kg)	84.7 ± 15.9	82.4 ± 14.8	0.603
BMI (kg/m^2)	28.5 ± 4.2	27.2 ± 4.0	0.256
Smokers (%)	5.7	3.8	1
Use of antihypertensive medication (%)	18.9	29.1	0.17

Values are partly given as mean ± SD
[a]RR (blood pressure according to Riva Rocchi)

Results

Three participants of the 54 enrolled subjects (27 in the milk drink group, 27 in the control drink group) dropped out due to changes in their medication and one participant due to severe illness. Fifty subjects, 25 in the milk drink group and 25 in the control group, completed the study. The ITT-population consisted of 53 patients (27 in the milk drink group, 26 in the control drink group). The demographic data are presented in Table 2. Except of age, all other anthropometric variables including systolic and diastolic blood pressure were comparable in the two groups. The patients in the milk drink group were younger compared to the placebo group (47.8 years ± 11.6 vs. 55.3 years ± 10.4; $p = 0.014$).

Both drinks were taken regularly every morning according to the information obtained from the patients and the drinks were overall well tolerated. In the first week two patients of each group reported about flatulence. Lifestyle, body weight, and dietary habits remained stable during the study (data not shown).

Effect on blood pressure

At baseline, systolic and diastolic blood pressure were comparable between the two groups (Table 2). Blood pressure values did not change during the course of the study in the milk drink group 144.1 ± 8.6/91.0 ± 5.5 mmHg at baseline vs. 143.7 ± 13.5/90.4 ± 6.5 mmHg after 12 weeks. In the control group systolic ($p = 0.0431$) and diastolic ($p = 0.0081$) blood pressure was significantly reduced 140.6 ± 11.7/90.3 ± 5.8 mmHg at baseline vs. 137.0 ± 14.4/87.7 ± 6.6 mmHg after 12 weeks (Fig. 1A, B). No differences were seen between the two groups. The analysis of the 24-h ambulatory blood pressure monitoring also revealed no changes before and after treatment between the groups (milk drink group 145.9 ± 11.0/94.4 ± 6.8 mmHg at baseline vs. 147.6 ± 15.3/95.8 ± 8.6 mmHg after 12 weeks and for the control group: 141.4 ± 13.6/91.5 ± 9.4 mmHg at baseline vs. 141.4 ± 12.9/91.2 ± 9.8 mmHg after 12 weeks) (Fig. 1C).

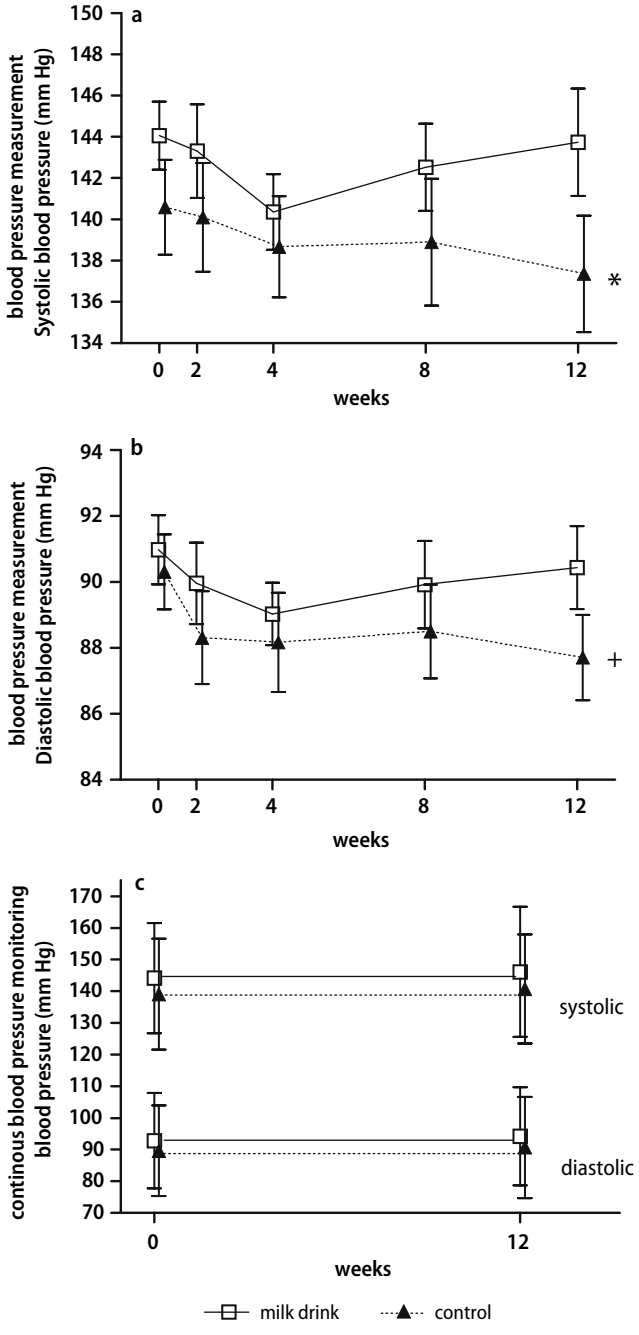

Fig. 1 Effect of a milk drink containing whey peptides vs. a control drink on systolic blood pressure (**A**), diastolic blood pressure (**B**), and ambulatory continuous blood pressure monitoring (**C**) during a 12-week administration. Data are means of the absolute changes vs. control and SD. *$P = 0.0431$ (week 12 vs. baseline); $^{+}P = 0.0081$ (week 12 vs. baseline)

Table 3 Effect of a milk drink supplemented with whey peptides on selected pro-inflammatory and anti-fibrinolytic parameters at baseline and after 12 weeks of treatment

	Milk drink group		Control group	
	Baseline	After treatment	Baseline	After treatment
CRP (mg/l)	2.23 ± 2.14	3.06 ± 4.25	2.31 ± 3.76	2.64 ± 4.73
IL-6 (ng/ml)	24.5 ± 23.8	26.6 ± 20.8	29.4 ± 29.3	32.3 ± 34.1
Leukocytes (per nl)	5.77 ± 1.05	5.65 ± 1.03	6.15 ± 2.17	6.40 ± 2.29
PAI-1 (U/ml)	5.3 ± 2.6	5.5 ± 2.8	4.9 ± 3.3	5.0 ± 3.7

Values are given as mean ± 95%-confidence interval. Number of samples in the groups ranged between 22 and 25

Effect on inflammatory and metabolic parameters

Due to the presumed ACE-inhibitory activity of the whey peptides contained in the milk drink we investigated whether an effect could be seen on inflammatory markers. CRP, IL-6 levels, and number of leukocytes did not change significantly, neither over the course of the study nor between the two groups. A potential effect on fibrinolysis was assessed by measuring PAI-1 activity. No significant changes could be seen neither over the course of the study nor between the two groups (Table 3). Furthermore, metabolic parameters such as fasting plasma glucose, blood lipids were also assessed with no effects found between the two groups. Also the HOMA-Index was unaffected. The concentrations at baseline and after 12 weeks of treatment are presented in Table 4.

Discussion

The results of this controlled clinical study clearly indicate that regular consumption of a milk drink containing whey peptides has no effect on blood pressure in mildly hypertensive individuals. This clinical data do not confirm the results of previous in vitro experiments which demonstrated an ACE-inhibiting activity of the whey powder.

Research during the last 20 years provided evidence that milk protein contains peptides with an inhibitory effect on ACE. Such studies established that casein is a source of ACE-inhibitory peptides also known as casokinins [10]. Quite a few casokinins are identified by now and intensively studied in vitro [11–17]. In vitro studies have shown that the major whey proteins, i.e., α-lactalbumin and β-lactglobulin also contain peptides with ACE-inhibiting activity. These whey-derived inhibitors are known as lactokinins [9].

While for casokinins many in vivo studies in rats and in humans [5, 6] exist, no human data are available for lactokinins. But, especially for functional food ingredients and nutraceuticals, it is very important to confirm in vitro findings in vivo. As for casokinins in vivo studies in spontaneously hypertensive rats (SHR) showed that the extent of ACE-inhibitory activity is not necessarily correlated with the anti-hypertensive activity. Maruyama et al. [12] described a casokinin with an IC_{50} value of 77 mM. Oral administration of 100 mg/kg led to a decrease by 13.0 mmHg of systolic blood pressure after 3 h. Another Japanese group used a casokinin with an IC_{50} value of 16 mM and found a decrease of systolic blood pressure to a similar extent, which is 13.6 mmHg after 3 h of oral administration of 100 mg/kg [14]. However, in the case of the casokinin Tyr-Lys-Val-Pro-Gln-Leu, which showed an IC_{50} value of 22 μM, no hypotensive effect was detectable after oral administration of 1 mg/kg in SHR after 6 h [17].

Previous in vitro tests of the whey powder used in the milk drinks of this clinical study had clearly demonstrated an ACE-inhibitory activity. An IC_{50} value at a dilution of 1:35 of the whey powder was

Table 4 Serum parameters of the ITT-population at baseline and after 12 weeks of treatment

	Milk drink group		Control group	
	Baseline	After treatment	Baseline	After treatment
Fasting plasma glucose (mg/dl)	90.6 ± 12.1	89.7 ± 12.4	86.6 ± 9.3	86.2 ± 13.6
Insulin (μU/ml)	11.5 ± 7.3	12.5 ± 9.0	8.8 ± 6.3	12.5 ± 10.3
HOMA-Index	2.40 ± 1.82	2.51 ± 2.49	1.70 ± 1.69	2.58 ± 2.85
Cholesterol (mg/dl)	205.5 ± 32.6	203.3 ± 41.0	205.5 ± 53.1	201.5 ± 43.4
HDL-Cholesterol (mg/dl)	58.3 ± 11.5	55.2 ± 11.5	59.8 ± 17.2	58.4 ± 14.7
LDL-Cholesterol (mg/dl)	119.7 ± 30.7	119.4 ± 37.2	116.5 ± 43.4	106.7 ± 32.3
Triglycerides (mg/dl)	137.5 ± 73.0	143.2 ± 77.7	145.5 ± 110.5	171.4 ± 119.4

Values are given as mean ± SD. Number of samples in the groups ranged between 23 and 26

measured. To verify this finding, this randomized, double blind, placebo-controlled clinical trial aimed at investigating the effect of the milk drink supplemented with whey peptides on blood pressure in mildly hypertensive subjects. To our surprise no significant effect on blood pressure could be detected with the test product.

One possible explanation for the missing effect could be degradation of the peptides by intestinal or plasma peptidases before they could exert an effect on blood pressure. Another explanation could be an insufficient resorption of the peptides in the milk drink although proven to be bioactive in vitro. Usually, pharmacological effects of ACE-inhibitors on blood pressure are apparent after 2 weeks of treatment. As we have chosen an intervention period of 12 weeks, it is rather unlikely that the exposure time was too short to see a significant effect.

Recent clinical studies revealed that administration of ACE-inhibitors also has some favorable effects on inflammatory markers. Chronic subclinical inflammation is currently discussed to play an important role in the pathogenesis of arteriosclerosis [19]. Therefore, selected inflammatory parameters were assessed to study potential anti-inflammatory effects. Neither CRP, IL-6 nor number of leukocytes was influenced by the milk drink. PAI-1-activity as an important parameter of fibrinolysis but also as inflammatory marker was equally unaffected. These findings are in agreement with the missing effect of the milk drink on blood pressure.

A possible limitation of the study was that only one single dose of whey proteins was applied. It cannot be excluded at the present time that a higher dosage may be required to exert a measurable biological effect in humans. Therefore, to fully examine the blood pressure lowering potential of whey proteins, a dose–response study may be appropriate. Unfortunately, it is currently not possible to examine other explanations such as bioavailability of the peptides as no appropriate methods are available to clarify this issue. It was also reported that whey protein-derived ACE-inhibitory activities are not as potent as synthetic anti-hypertensive drugs such as captopril [9]. Therefore, it still remains an open question as to whether whey peptides are potent enough even at higher doses to mediate a significant anti-hypertensive effect in man.

In conclusion, daily consumption of a milk drink supplemented with whey peptides presumed to contain ACE-inhibiting peptides did not significantly affect blood pressure and/or inflammation markers in mildly hypertensive subjects. Nevertheless, additional human studies are needed to investigate the potential of lactokinins in the prevention and early treatment of hypertension and subsequent cardiovascular disease.

Acknowledgements This study was funded by the Unternehmensgruppe Theo Müller, Aretsried, Germany.We also wish to thank Prof. W. Koenig, University Hospital Ulm, for performing the analysis of CRP.

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