

Dairy intake, blood pressure and hypertension

Observational and intervention studies

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Abstract

Background

Worldwide the prevalence of hypertension is increasing rapidly, which calls for effective public health measures. Whether dairy intake could play a role in reducing population blood pressure is not yet clear. The objective of this thesis was to examine the association of dairy intake with blood pressure level and incident hypertension in the Dutch population, and to assess the blood pressure effect of two 'promising' dairy components, i.e. lactotriptides (IPP and VPP) and *cis-9, trans-11* conjugated linoleic acid (CLA).

Methods

Intakes of total dairy and types of dairy were examined in relation to blood pressure level in 21,553 Dutch adults aged 20-65 y from the MORGEN study. The risk of hypertension was examined in 3454 of these participants with 5 y of follow-up and in 2245 older Dutch adults from the Rotterdam study with 6 y of follow-up. The blood pressure effect of lactotriptides was assessed in an 8-week, randomized controlled trial in 135 middle-aged Dutch subjects with elevated blood pressure. The effect of a high dose of *cis-9, trans-11* CLA on blood pressure was studied in a 9-week, randomized cross-over trial in 61 young, normotensive Dutch subjects.

Results

Blood pressure levels were not consistently related to overall dairy intake or intake of specific dairy foods in the general Dutch population (MORGEN study). Longitudinal analyses in the MORGEN study and the Rotterdam study showed a ~20% reduced risk of hypertension in subjects who consumed more than 150 mL (~1 serving) of low-fat dairy per day, whereas other dairy foods were not consistently associated with incident hypertension. Blood pressure was not affected by intervention with lactotriptides. The mean difference (95% confidence interval) in systolic blood pressure response between the treatment and control group was 2.8 mm Hg (-2.6, 8.2) for lactotriptides obtained by fermentation, -0.5 mm Hg (-6.0, 5.0) for lactotriptides obtained by enzymatic hydrolysis, and 1.6 mm Hg (-3.9, 6.9) for synthetic lactotriptides ($p = 0.46$). With regard to intervention with *cis-9, trans-11* CLA, systolic blood pressure changed -0.1 mm Hg (-1.49, 1.27) compared to control treatment (oleic acid), which was not statistically significant ($p = 0.87$).

Conclusion

The findings presented in this thesis suggest that low-fat dairy products may reduce the risk of hypertension. However, it should be noted that evidence has mainly been derived from observational studies and controlled intervention studies are needed to

confirm these findings. With regard to dairy components, we conclude that lactotriptides and CLA are unlikely to play an important role in blood pressure regulation. Current dietary guidelines in countries like the US and the Netherlands include 2-3 servings of low-fat or fat-free dairy per day, which is mainly based on the prevention of osteoporosis by ensuring an adequate intake of calcium. Based on the findings presented in this thesis, and evidence from the scientific literature, there is at present no need to adapt these recommendations for the purpose of hypertension prevention.

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Chapter 1

Introduction

Blood pressure and cardiovascular risk

Hypertension is an important public-health challenge worldwide. The definition of hypertension and classification of blood pressure levels is presented in **Table 1.1**. In 2000, 25% of the world's adult population had hypertension and this is expected to increase to 29% in 2025¹. In the Netherlands, around 75% of the adults have suboptimal blood pressures (i.e. 120/80 mm Hg or higher), comprising roughly 25% with hypertension, 25% with 'high-normal' blood pressure and 25% with 'normal' blood pressure (as defined in **Table 1.1**)². Blood pressure is a strong, independent and modifiable risk factor for cardiovascular and renal diseases³. Systolic blood pressure nowadays is considered a more powerful predictor of clinical endpoints than diastolic blood pressure, especially after the age of 55 y⁴. There is no evidence for a threshold effect, i.e. the risk of cardiovascular morbidity and mortality increases from blood pressure levels as low as 115 mm Hg systolic upward^{5, 6}. Suboptimal blood pressure is responsible for 54% of stroke and 47% of ischemic heart disease worldwide⁷; about half of this burden can be attributed to hypertension, and the remainder to lower levels of blood pressure. In view of the continuing epidemic of blood pressure-related diseases, effective public health approaches that lead to population-wide reductions in blood pressure are warranted even among those who do not have hypertension.

Table 1.1 Definition of hypertension and classification of blood pressure levels¹

Category	Systolic blood pressure (mm Hg)		Diastolic blood pressure (mm Hg)
Optimal	<120	and	<80
Normal ²	120-129	and/or	80-84
High Normal ²	130-139	and/or	85-89
Grade 1 (mild) hypertension ³	140-159	and/or	90-99
Grade 2 (moderate) hypertension ³	160-179	and/or	100-109
Grade 3 (severe) hypertension ³	≥180	and/or	≥110
Isolated systolic hypertension ³	≥140	and	<90

¹ According to 2007 ESH/ESC guidelines on hypertension⁸.

² 'Normal' and 'high normal' combined are considered 'prehypertension' according to 2003 JNC 7 guidelines on hypertension⁹.

³ Or use of antihypertensive medication.

The worldwide prevalence of hypertension is increasing rapidly, which calls for effective public health measures throughout the entire range of blood pressure.

Dietary prevention strategies

It has been estimated that as much as 80% of cardiovascular events could be prevented by a healthy diet and lifestyle^{9, 10}. International guidelines for the management of hypertension include lifestyle modifications for persons with high-normal blood

pressure and hypertension. Well-established dietary factors that lower blood pressure are weight reduction in overweight and obese individuals, reduced salt intake, moderation of alcohol consumption (among those who drink), and increased potassium intake^{11, 12}. More recently, interest has shifted towards the total dietary pattern, rather than focusing on individual nutrients. A hallmark study in this respect is the Dietary Approaches to Stop Hypertension (DASH) trial¹³. In this well-controlled feeding study, blood pressure in 459 untreated mildly hypertensive US individuals was substantially reduced by a diet that emphasized fruits, vegetables, and low-fat dairy products and contained less saturated fat (amongst other dietary modifications). Because of the multifactorial intervention, it is not clear to what extent the inclusion of (low-fat) dairy contributed to the 'DASH' effect. US health authorities now recommend a DASH-type dietary pattern, including 2-3 daily servings of low-fat dairy products as an effective non-pharmacological measure to lower blood pressure¹¹.

Dietary prevention strategies are important for maintaining a healthy blood pressure. Whether intake of dairy products could play a role in reducing population blood pressure remains to be established.

Dairy and blood pressure: observational evidence

Since the publication of the DASH trial, the association of dairy intake with blood pressure and incident hypertension has been examined in different populations, both cross-sectionally¹⁴⁻¹⁸ and prospectively^{15, 19-25} (**Table 1.2**). In 7 out of 12 studies total dairy intake was inversely related to blood pressure or risk of hypertension^{14, 16, 17, 20, 21, 23, 25}, whereas other studies did not show consistent associations for total dairy intake^{15, 18, 19, 22, 24}. Apart from chance, this may be attributed to heterogeneity of study populations (e.g. age, race, blood pressure level, overweight status), study design (cross-sectional, short-term prospective, and long-term prospective studies), classification and range of dairy intake, differences in 'background' diet, and correction for major confounders (e.g. dairy-related dietary factors, physical activity).

Little is known about the possible influence of dairy groups (e.g. low-fat dairy, high-fat dairy, fermented dairy) as well as individual dairy products (e.g. cheese, yogurt) on blood pressure. Most of the dairy products in the DASH diet were in the form of 1% low-fat or fat-free milk (74%) and low-fat or fat-free yogurt (18%)²⁶. Several observational studies reported findings separately for low-fat and high-fat dairy^{18-21, 24, 25} (**Table 1.2**). Three studies reported an inverse association for low-fat but not for high-fat dairy products^{19, 24, 25}, whereas this was not confirmed by others^{18, 20, 21}. In about half of the studies the association of specific products with blood pressure or hypertension was examined^{17, 18, 21-23, 25}, but no consistent relationships emerged from these data.

Observational studies generally show inverse associations between dairy intake and blood pressure or hypertension, although findings are not conclusive. Little is known about specific types of dairy foods in relation to blood pressure.

Dairy and blood pressure: experimental evidence

Intervention studies of dairy intake and blood pressure are scarce. Often cited in this respect is the above mentioned DASH trial¹³. This study showed that blood pressure reductions were more pronounced when low-fat dairy products were added to a healthy fruits-and-vegetables diet (apart from other dietary changes). It should be emphasized, however, that the DASH trial was not designed to assess the effect of single food groups. The role of dairy foods in blood pressure control can therefore not be established on basis of this trial. We identified 7 trials in which dairy formed part of the dietary intervention (**Table 1.3**). Of these, only 2 studies aimed to assess the effect of dairy per se on blood pressure, showing no clear beneficial effect^{27, 28}. In a cross-over trial by Bierenbaum et al. dairy products (yogurt, cottage cheese, and milk) did not lower blood pressure compared to orange juice in 50 normotensive US men and women²⁸. Barr et al. showed no effect on blood pressure of an increase of 3 glasses of low-fat milk per day in 204 normotensive US adults aged 55 y and over²⁷. Other trials focused on components in a dairy matrix (e.g. minerals)²⁹⁻³², or included dairy as part of a changed dietary pattern¹³. Dairy minerals (calcium, potassium, magnesium) were significantly shown to lower systolic blood pressure by 3 mm Hg in 60 normotensive Dutch subjects³¹. A high-calcium diet by manipulating dairy intake, however, did not significantly lower blood pressure in 13 mildly untreated hypertensive US men³⁰. In a randomized double-blind cross-over trial, a high-calcium milk enriched with potassium had a small hypotensive effect compared to skim milk or high-calcium skim milk in 38 healthy Caucasian adults aged 40 y and over²⁹, whereas increasing the potassium content of skimmed milk by 750 mg did not affect blood pressure in 113 mildly hypertensive subjects in a well-controlled randomized trial by Van Mierlo et al.³².

Randomized controlled trials exclusively focusing on dairy are scarce and data are insufficient to draw conclusions.

Dairy components and blood pressure

Dairy products are a main source of calcium, accounting for about 70% of the total daily calcium intake in the Netherlands³³. The importance of dietary calcium in the prevention and treatment of hypertension was first suggested in 1984³⁴ and has been examined extensively. Several meta-analyses of clinical trials showed that the effect of calcium on blood pressure is well-established, but relatively small with reductions in systolic blood pressure of 1-2 mm Hg for calcium doses of 400 to 2000 mg/d³⁵⁻³⁸. It has been suggested that the impact of calcium on blood pressure may be greater in persons

with a low habitual intake of dietary calcium^{37, 38}. This would imply that in countries like the Netherlands, with an average intake of calcium of ~1000 mg/d³³, there is little need to advocate higher calcium intakes to reduce population blood pressure. On the contrary, in countries with less calcium in the diet (e.g. US) recommendations to increase calcium via dairy intake may be more important for the prevention of hypertension³⁹. When reviewing intervention studies on calcium and blood pressure, there seems to be greater consistency for calcium-rich foods than non-dietary calcium supplements³⁷. This would emphasize the importance of the food matrix or total dietary pattern. Because vitamin D is important for calcium absorption, it has been suggested that the effect of calcium on blood pressure may depend on vitamin D status^{25, 40}. Other minerals that are present in dairy foods have also been inversely related to blood pressure. Blood pressure reductions from 2 to 6 mm Hg systolic have been found in trials of potassium supplementation⁴¹⁻⁴³ and weaker effects (~1 mm Hg systolic) have been found for supplemental magnesium^{39, 44}. Apart from dairy minerals with a blood pressure lowering potential, dairy products (mainly cheese) also contain sodium which is a well-established risk factor for blood pressure¹¹.

A relatively new line of research focuses on the blood pressure-lowering potential of milk-derived bioactive peptides, which are amino acid sequences with biological activity that are embedded in milk protein. A wide range of activities of these peptides have been described, including antihypertensive properties, immunomodulatory and opioid properties, enhancement of mineral absorption and localized effects on the gut⁴⁵. Latent bioactive peptides can be released from protein during food processing, e.g. via fermentation of casein by *Lactobacillus helveticus* or enzymatic hydrolysis by *Aspergillus oryzae* protease. Fermented milks and casein hydrolysates have been shown to lower blood pressure in a number of trials in (mildly) hypertensive subjects, mainly from Japan and Finland^{46, 47}. So far, reported results have been promising; two meta-analyses of randomized controlled trials showed a mean reduction in blood pressure of 5 mm Hg systolic and 2 mm Hg diastolic^{46, 47}. However, results have not yet been confirmed in other populations. Inhibition of the angiotensin-converting enzyme (ACE) has been proposed as an underlying mechanism for the blood pressure lowering effect of milk-derived peptides. ACE plays a key role in blood pressure regulation by generating the vasoconstricting angiotensin II from angiotensin I, and inactivating bradykinin which is a vasodilator. Isoleucine-Proline-Proline (IPP) and Valine-Proline-Proline (VPP), two peptides from milk casein, are considered promising ACE-inhibiting bioactive peptides. However, although ACE-inhibiting properties of fermented milk or its peptides have been demonstrated in *in vitro* and animal models^{48, 49}, recent data in humans suggest that the plasma concentration of ACE-inhibiting peptides are far below the effective concentration for plasma ACE-inhibition⁵⁰.

Another dairy component that has recently attracted much attention is *cis-9, trans-11* conjugated linoleic acid (CLA). *Cis-9, trans-11* CLA is produced in the rumens of cows, sheep and other ruminant animals through partial hydrogenation of unsaturated fatty acids from the feed by bacteria. It can also be formed from vaccenic acid in animals and in humans⁵¹. It is consumed by humans in foods and as supplements. In general, supplements consist of 50:50 mixtures of the isomers *cis-9, trans-11* CLA and *trans-10, cis-12* CLA or mixtures of more isomers. CLA in dairy products consists of over 90% of the *cis-9, trans-11* CLA isomer, although absolute amounts are small. Favorable effects of CLA on health, for example weight reduction, insulin sensitivity and blood lipid profiles have been reported in animal studies⁵². In addition, CLA was found to lower blood pressure in several rat models⁵³⁻⁵⁵. On the other hand, CLA is a *trans* fatty acid and harmful effects in the cardiovascular system cannot be excluded. Little is known about the effect of CLA on human blood pressure.

There are several hypotheses on how dairy may lower blood pressure, but the underlying mechanisms are largely unknown. Calcium is considered the main antihypertensive component in dairy, although its effect is relatively small. Two dairy components that have recently attracted much attention are lactotripeptides (IPP and VPP) and cis-9, trans-11 conjugated linoleic acid (CLA). Their role in blood pressure regulation, however, has not been established.

Dairy intake in the Netherlands

The Netherlands is a country with a relatively high average dairy intake from a wide variety of products. Dairy products form part of the Dutch traditional diet and 70-80% is consumed at home³³. Around 75% is consumed at three fixed moments during the day, i.e. milk and milk products and cheese during two bread meals (breakfast and lunch) and dairy desserts after dinner. In the Netherlands, current recommendations for the daily intake of dairy products are 450-650 mL of milk and milk products and 20-30 g of cheese from the age of 19 y onwards⁵⁶. With an average daily intake of 350-400 mL of milk and milk products most adults do not meet this recommendation, whereas for cheese the recommended intake is achieved³³.

In the Netherlands, milk and milk products and cheese contribute substantially to daily intake of calcium (69-75%), vitamin B2 (riboflavin; ~50%), vitamin B12 (cobalamin; ~35%), phosphorus (~35%), protein (~25%), zinc (~25%), potassium (~18%), magnesium (~18%) and sodium (~15%)³³. In the Netherlands, dairy products (except for margarine) are not fortified with vitamin D, but they still contribute significantly to the total vitamin D intake (~10%)³³. Contributions to the intake of energy, fat and saturated fat are ~15%, ~18% and ~28% respectively³³. The Dutch Nutrition Center emphasizes the use of (semi-)skimmed dairy products⁵⁶. During the 1998 Dutch National Food Consumption Survey the relative contributions of full fat, semi-skimmed and skimmed

milk to the total consumption of milk and milk products were 18%, 53% and 29% respectively (**Figure 1.1**)³³. It has been estimated that the contribution of semi-skimmed milk has now increased to about 85%.

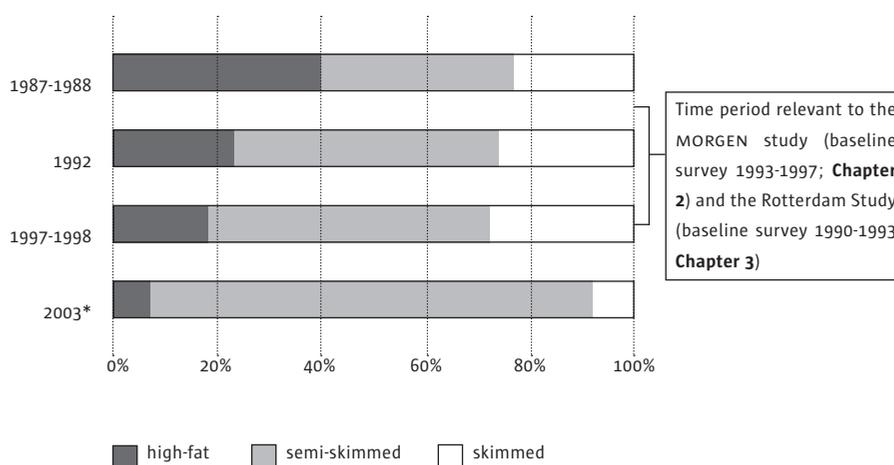


Figure 1.1 Contribution of high-fat (>3.5 g/100 g), semi-skimmed (<2 g/100 g) and skimmed (<0.5 g/100 g) milk products to the total consumption of milk and milk products in the Dutch population from 1988 to 2003^{33, 57-59}.

*The food consumption survey of 2003 has been conducted only among young adults (19-31 y).

Although dairy products are commonly consumed in the Netherlands, information on the association between dairy intake and blood pressure is scarce for the Dutch situation. To our best knowledge, this association has only been examined in a population-based cohort of 2064 Dutch men and women aged 50-75 y (Hoorn Study). The intake of dairy, in particular dairy desserts, milk and yogurt showed a modest inverse association with blood pressure¹⁸, but not with change in blood pressure²² (**Table 1.2**).

In the Netherlands, where dairy products are commonly consumed, information on the association between dairy intake and blood pressure is hardly available. The wide range of dairy intake and large variety of dairy foods that are consumed allows detailed examination of different dairy foods in relation to blood pressure and incidence of hypertension.

Rationale and outline of the thesis

The worldwide prevalence of hypertension is increasing rapidly, which calls for effective public health measures. The Dietary Approaches to Stop Hypertension (DASH) trial showed that blood pressure reductions were more pronounced when low-fat dairy (among other dietary changes) was added to a fruits-and-vegetables diet. Whether intake of dairy products could play a role in reducing population blood pressure,

however, is not clear. Observational studies examining the association between dairy intake and blood pressure or hypertension generally suggest an inverse association, although data are not conclusive. Little is known about the effect of different types of dairy foods in relation to blood pressure. The Netherlands is a country where a wide variety of dairy products is consumed. This allows detailed examination of the association of total dairy and specific types of dairy foods with blood pressure over a broad range of intake. Intervention studies on dairy intake and blood pressure are scarce, but a fair amount of research on the blood pressure lowering potential of specific dairy components has been done. Calcium is considered one of the main nutrients responsible for a (possible) beneficial effect of dairy on blood pressure, although the size of effect is relatively small. Two other dairy components that have recently attracted much attention are lactotriptides (IPP and VPP) and *cis-9, trans-11* conjugated linoleic acid (CLA). Promising results for IPP and VPP have been shown in Japanese and Finnish subjects with elevated blood pressure. The efficacy of these two lactotriptides in other populations, including Dutch hypertensive subjects, has not yet been established. CLA has recently attracted much interest because of possible favorable effects on health, including reduction of blood pressure in several rat models. On the other hand, CLA is a *trans* fatty acids and harmful effects in the cardiovascular system cannot be excluded. Whether CLA could influence human blood pressure is not yet clear.

The research described in this thesis was initiated to further investigate the role of dairy foods in relation to blood pressure and incident hypertension in the Dutch population (outlined in **Figure 1.2**). The research objectives were:

- [1] to examine the association of dairy intake with blood pressure level and incident hypertension in a middle-aged (**chapter 2**) and older population (**chapter 3**),
- [2] to focus on specific types of dairy foods in relation to hypertension in these populations (**chapters 2 and 3**), and
- [3] to study the effect of two specific dairy components on human blood pressure, i.e. lactotriptides (IPP and VPP; **chapter 4**) and CLA (**chapter 5**).

In **chapter 6**, the findings of the research described in this thesis are discussed, possible underlying mechanisms are reviewed and findings are put into public health perspective.

Table 1.2 Observational studies of dairy intake, blood pressure and hypertension by year of publication

Author, year (study)	Design	Population	Habitual BP (mm Hg)	Habitual dairy intake	Result*	p-value	Statistical adjustment	Remarks
Pereira, 2002 (CARDIA) ²¹	coh, 10y follow-up	3157 US black and white adults aged 18-30y Mean age 25y	nm	Q1: 0-10 times/wk Q5 ≥35 times/wk, based on 2 dietary histories; average of y 0 and 7	Cumulative incidence of HT <i>In overweight individuals:</i> Q1: 22.9% vs Q5: 8.7% <i>In lean individuals:</i> Q1: 10.3% vs Q5: 5.8%	p-trend <0.001 0.06	Age, sex, race, total en, study center, BMI	Study focused on dairy intake and the insulin resistance syndrome. Inverse association for both reduced-fat and high-fat dairy products, milk and milk products. Not significant for cheese, butter, desserts and yogurt.
Alonso, 2005 (SUN) ¹⁹	coh, 2y follow-up	5880 Spanish university graduates >20y Mean age 37y	Free of HT	Q1: 156 g/d Q5: 799 g/d Low-fat dairy: Q1: 3 g/d Q5: 615 g/d, based on FFO	<u>HR total dairy:</u> Q5 vs Q1: 0.75 (0.45, 1.27) <u>HR low-fat dairy:</u> Q5 vs Q1: 0.46 (0.26, 0.84)	p-trend 0.12 0.02	Age, sex, BMI, PA, alcohol, smoking, hypercholesterolemia, intake of total en, fruit, vegetables, fiber, caffeine, Mg, Na, K, MUFA, SFA	Significant association only for low-fat dairy or Ca from low-fat dairy, not for total dairy, whole-fat dairy or total Ca intake. Not significantly different between men/women, younger/older, and lean/obese persons.
Azadbakht, 2005 (Tehran Lipid and Glucose Study) ¹⁴	cs	827 Iranian adults aged 18-74y	nm	Q1: <1.7 servings/d Q4: ≥3.1 servings/d, based on FFO (1serving ~240g)	<u>OR for EBP (≥130/85 mm Hg):</u> Q4 vs Q1: 0.76 (0.62, 0.91)	p-trend <0.02	Age, sex, smoking, PA, BMI, WHR, total en, en% fat, meat, fruit, vegetables, grains, antihypertensive drugs, estrogen	Further adjustment for Ca and protein slightly attenuated the association between dairy and EBP. Specific dairy groups were not examined.
Moore, 2005 (Framingham Children's Study) ²⁰	coh, 12y follow-up	95 US children aged 3-6y	~94/53	Low: <2 servings/d High: >2 servings/d, based on repeated 3-day food diaries	<u>Yearly change in BP:</u> SBP Low: 2.95 ± 0.19 mm Hg SBP High: 2.13 ± 0.25 mm Hg DBP Low: 0.74 ± 0.13 mm Hg DBP High: 0.58 ± 0.16 mm Hg	nm	Baseline BP, PA, intake of Mg, Na, fruit, vegetables	Additional adjustment for change in BMI did not materially change the findings. No consistent beneficial effect of consuming predominantly low-fat dairy products.
Steffen, 2005 (CARDIA) ²³	coh, 15y follow-up	4304 US black and white adults aged 18-30y Free of EBP, no antihypertensive medication	111/73	2.4 times/d Q1: <1.1 times/d Q5: >3.4 times/d, based on 2 dietary histories; average of y 0 and 7	<u>HR for EBP (≥130/85 mm Hg):</u> Q5 vs Q1: 0.85 (0.67, 1.08)	p-trend 0.06	Age, sex, race, education, center, en intake, PA, alcohol, smoking, vitamin supplements, intake of plant food, meat, poultry, eggs, fish, seafood	Further adjustment for explanatory nutrients (Na, Ca, SFA, Mg, K, fiber) and baseline BP, BMI, fasting insulin further attenuated association. Inverse association for milk and dairy desserts, but not for cheese or yogurt.

Author, year (study)	Design	Population	Habitual BP (mm Hg)	Habitual dairy intake	Result*	p-value	Statistical adjustment	Remarks
Djoussé, 2006 (NHLBI Family Heart Study) ¹⁶	cs	4797 US men and women Mean age 52y	~118/69	Q1: 0.5 servings/d Q4: 3.5 servings/d, based on FFQ (1 serving: ~240 g)	Prevalence OR for HT: Q4 vs Q1: 0.64 (0.46, 0.90)	p-trend 0.01	Age, sex, field center, education, BMI, total en, linolenic acid, SFA, MUFA, Na, K, caffeine, fiber, fruit, vegetables, Mg, alcohol, smoking, history of CHD and DM	Association between dairy and HT was independent of dietary Ca and mainly among persons consuming less SFA. Dairy consumption was inversely associated with SBP, but not with DBP. Specific dairy groups were not examined.
Ruidavets, 2006 (MONICA) ¹⁷	cs	912 French men aged 45-64y Mean age 55y	~139/86	Q1: ~93 g/d Q5: >335 g/d, based on 3-d food record	<u>Difference in BP level</u> Q5 vs Q1: SBP: -3.94 ± 2.37 mm Hg DBP: -1.60 ± 1.52 mm Hg	p-trend 0.08 0.20	Age, center, PA, smoking, alcohol, BMI, antihypertensive and dyslipidaemia medication, dieting, total en, Na, Mg, Ca, diet, quality index	Dairy products and dietary Ca were both significantly and independently associated with low levels of SBP. Dairies without butter, milk, and fresh cheese were also inversely associated with SBP. Associations were stronger in subjects not treated for HT.
Dauchet, 2007 (SU.VI.MAX) ¹⁵	cs, 5y follow-up	4652 French subjects of study aged 35-63y Follow-up in 2341 subjects	123/79	Q1: 83 g/d Q4: 460 g/d, based on repeated 24-h dietary records	<u>Difference in BP level</u> Q4 vs Q1: SBP: -1.2 mm Hg ($-2.3, -0.1$) DBP: -1.1 mm Hg ($-1.8, -0.4$) <u>Difference in 5y BP change</u> Q4 vs Q1: SBP: $+0.1$ mm Hg ($-1.2, 1.5$) DBP: -0.2 mm Hg ($-1.2, 0.7$)	p-trend 0.16 <0.004	Age, sex, treatment, completion of dietary records, smoking, alcohol, PA, education, BMI, total en, Na, fruit, vegetables, Keys score (and baseline BP for coh. analysis)	Association between dairy and baseline BP only in men. Specific dairy groups were not examined.
Snijder, 2007 (Hoorn Study) ¹⁸	cs	1896 Dutch adults aged 50-75y Mean age 62y	~136/82	Median: 4 servings/d Q1: 0-3 servings/d Q4: 6-17 servings/d, based on FFQ (1 serving ~150 g)	<u>BP per serving of total dairy:</u> SBP: -0.23 ± 0.22 mm Hg DBP: -0.31 ± 0.12 mm Hg <u>BP per serving of low-fat dairy:</u> SBP: -0.06 ± 0.25 mm Hg DBP: -0.22 ± 0.14 mm Hg	0.29 0.01 0.83 0.11	Age, sex, total en, PA, alcohol, smoking, income, education, fiber, antihypertensive medication	Modest inverse associations with BP were observed for dairy desserts, milk, and yogurt. Cheese was not related to BP. Associations were stronger in hypertensive subjects.

Author, year (study)	Design	Population	Habitual BP (mm Hg)	Habitual dairy intake	Result*	p-value	Statistical adjustment	Remarks
Wang, 2008 (Women's Health Study) ²⁵	coh, 10y follow-up	28,886 female US health professionals aged $\geq 45y$ Mean age 54y	Free of HT	Q1: 0.6 servings/d Q5: 3.7 servings/d Low-fat: Q1: 0.1 serving/d Q4: 2.7 serving/d based on FFQ	RR for total dairy: Q5 vs Q1: 0.86 (0.79, 0.93) RR for low-fat dairy: Q5 vs Q1: 0.89 (0.82, 0.96) No significant association between high-fat dairy and HT.	p-trend 0.0003 0.001	Age, race, total en, treatment, smoking, alcohol, PA, postmenopausal status, multivitamins, BMI, DM, hypercholesterolemia, fruits, vegetables, whole grains, red meat	No interaction with BMI, alcohol, PA or baseline BP. Further adjustment for dietary Ca attenuated association, while dietary vitamin D did not. Dietary Ca and vitamin D were also inversely associated with HT, while supplements were not. Skim milk, sherbet, yogurt and cottage cheese (main low-fat products) not clearly related to HT risk.
Snijder, 2008 (Hoorn Study) ²²	coh, 6y follow-up	1124 Dutch adults aged 50-75y Mean age 60y	~133/82	Q1: 0.3 servings/d Q4: 6-17 servings/d based on FFQ (1 serving ~150 g)	6y change in BP per serving: SBP: $+0.27 \pm 0.25$ mm Hg DBP: -0.002 ± 0.149 mm Hg	0.29 0.99	Age, sex, total en, baseline BP, alcohol intake, smoking, PA	Also no association with low-fat dairy, high-fat dairy, yogurt, milk, desserts or cheese. Also no significant associations in subjects with low Ca intake (<700 mg/d).
Toledo, 2008 (PREDIMED) ²⁴	cs, 1y follow-up	2290 older Spanish adults, high CVD risk (including 1845 with HT) Mean age ~67y	~150/84	Q1: 262 g/d Q5: 665 g/d Low-fat: Q1: 3 g/d Q5: 632 g/d, based on FFQ	BP level low-fat dairy Q5 vs Q1: SBP: -3.7 mm Hg ($-6.5, -0.9$) DBP: -1.7 mm Hg ($-3.2, -0.3$) Difference in 1y change in BP: Q5 vs Q1: SBP: -4.2 mm Hg ($-6.9, -1.4$) DBP: -1.8 mm Hg ($-3.2, -0.4$)	p-trend 0.05 0.13 0.01 0.09	Age, sex, center, BMI, PA, smoking, hyperlipidaemia, DM, total en, alcohol, Na, non-dairy K, Ca, Mg, and protein intake, SFA, MUFA, fiber, fruit, vegetables, use of defined medication	High-fat dairy not associated with BP level or change in BP. Results for total dairy were not reported.

Abbreviations: Q, either quartile (Q4) or quintile (Q5); coh, cohort; cs, cross-sectional; mm, not mentioned; BP, blood pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; EBP, elevated blood pressure; HT, hypertension; CHD, coronary heart disease; CVD, cardiovascular disease; DM, diabetes; WHR, waist-to-hip ratio; BMI, body mass index; PA, physical activity; FFQ, food-frequency questionnaire; en, energy; en%, percentage of energy intake; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; Study acronyms: CARDIA, Coronary Artery Risk Development in Young Adults; SUN, Seguimiento Universidad de Navarra; NHLBI, National Heart Lung and Blood Institute; MONICA, Monitoring of trends and determinants in Cardiovascular disease; PREDIMED, Prevención con Dieta Mediterránea.

* Shown are relative risks (RR), hazard ratios (HR), odds ratios (OR) or mean blood pressure values based on the multivariate-adjusted model, along with 95% confidence intervals between parentheses, standard deviations or standard errors.

Table 1.3 Intervention studies of dairy and blood pressure by year of publication

Author, year	Design	Subjects	Initial BP, mm Hg	Intervention	Results	P-value	Remarks
Bierenbaum, 1988 ²⁸	r, x 8-wk	50 normotensive US adults	nm	8 oz yogurt, 4 oz cottage cheese, and 16 oz milk/d vs 32 oz orange juice/d	Dairy vs orange juice: SBP: 115 vs 117 mm Hg DBP: 78 vs 79 mm Hg	nm nm	No statistical comparisons between intervention with dairy and orange juice were reported (only within group changes).
Van Berenstein, 1990 ³¹	r, p, db 6-wk	60 normotensive Dutch female students (19-23y)	~114/63	1L normal milk/d (1180 mg Ca, 1650 mg K, 110 mg Mg) vs 1L 'mineral-poor' milk/d (95 mg Ca, 580 mg K, 10 mg Mg)	<u>Normal milk vs 'mineral-poor' milk:</u> SBP: -5.1 vs -2.2 mm Hg No effect on DBP	0.03	Trial was performed against a low-Ca (<500 mg) background diet.
Kynast-Gales, 1992 ³⁰	r, x 4-wk	13 Caucasian men (46-75y), not using antihypertensive medication	136/83	High (1,500 mg) vs low (400 mg) Ca diet by manipulation of dairy intake	<u>High-Ca vs low-Ca diet:</u> SBP: 137 vs 133 mm Hg DBP: 84 vs 83 mm Hg	NS NS	Other minerals (Mg, P, K, Na) also different between treatments.
Appel, 1997 ¹³	r, p 8-wk	459 US adults (~45y), not using antihypertensive medication	131/85	Combination diet (fruits, vegetables, low-fat dairy, reduced SFA and total fat) vs fruits-and-vegetables diet vs 'standard US' control diet	<u>Combination diet vs control:</u> SBP: -5.5 mm Hg DBP: -3.0 mm Hg <u>Fruits-and-vegetables diet vs control:</u> SBP: -2.8 mm Hg DBP: -1.1 mm Hg	<0.001 <0.001 <0.001 0.07	The trial was not designed to assess the effect of single nutrients or foods.
Hilary Green, 2000 ²⁹	r, x, db 4-wk	38 Caucasian men and women aged >40y, not using antihypertensive medication	~125/77	480 ml high-Ca skim milk (1050 mg Ca, 855 mg K)/d vs high-Ca, K-enriched skim milk (1040 mg Ca, 1585 mg K)/d vs 'normal' skim milk (720 mg Ca, 885 mg K)/d (i.e. control)	<u>High-Ca, high-K vs high-Ca vs control:</u> SBP: 117 vs 122 vs 122 mm Hg DBP: 76 vs 75 vs 76 mm Hg	nm nm	No statistical comparisons between interventions are reported (only within group changes).

Author, year	Design	Subjects	Initial BP, mm Hg	Intervention	Results	P-value	Remarks
Barr, 2000 ²⁷	r, p, o 12-wk	204 men and women (~65y) with low dairy intake	~127/77	Skim or 1% milk 3 glasses/d vs maintaining usual diet	High milk vs low milk: SBP: -2 vs -3 mm Hg* DBP: -1 vs -1 mm Hg*	NS	Changes from baseline and treatment effect are not reported.
Van Mierlo, 2008 ³²	r, p, db 8-wk	113 untreated mildly hypertensive Dutch subjects	~144/86	Fortified skim milk + 1500 mg K (high-K) vs fortified skim milk + 750 mg K (low-K) vs Placebo (water)	High-K vs Low-K vs control: SBP: -4.6 vs -4.5 vs -5.1 mm Hg Also no effect on DBP and ambulatory BP.	NS	SBP declined by ~4 mm Hg during the trial in the placebo group and the two treatment groups.

Abbreviations: nm, not mentioned; r, randomized; p, parallel; x, cross-over; o, open; db, double-blind; BP, blood pressure; SBP, systolic blood pressure;

DBP, diastolic blood pressure; NS, not statistically significant (p > 0.05); Ca, calcium; Mg, magnesium; P, potassium; Na, sodium; SFA saturated fatty acids.

*Best guess based on implicit data.

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Chapter 2

Dairy intake, blood pressure and incident hypertension in a general Dutch population

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Abstract

Diet and lifestyle are important for maintaining a healthy blood pressure (BP). The role of dairy in the prevention of hypertension, however, is not yet clear. We studied the relation of dairy intake with BP in 21,553 Dutch participants aged 20-65 y who did not use antihypertensive medication. In addition, the risk of developing hypertension was examined in 3454 of these participants with 5 y follow-up. Dairy consumption was assessed at baseline (1993-1997) using a semiquantitative FFQ that included 178 foods and beverages. Baseline BP and odds ratios (OR) (95% CI) for incident hypertension were calculated in categories of energy-adjusted dairy intake with adjustment for age, sex, socioeconomic status, BMI, smoking, alcohol use and dietary intakes.

Participants had a median intake of 344 g/d (~2.3 servings) for total dairy and 174 g/d (~1.2 servings) for low-fat dairy. Mean BP was 120/76 mm Hg. Intake of total dairy, specific dairy groups (i.e., low-fat, high-fat, fermented) and dairy products (i.e., cheese, yogurt) were not consistently related to BP. Of 3454 participants who were followed, 713 developed hypertension. The risk of hypertension tended to be inversely related to low-fat dairy intake, with multivariate OR (95% CI) of 1.00, 0.78 (0.61, 1.00), 0.81 (0.63, 1.03) and 0.82 (0.64, 1.06; P-trend: 0.24) in consecutive quartiles. We conclude that variations in BP in a general middle-aged Dutch population cannot be explained by overall dairy intake. A beneficial effect of low-fat dairy on risk of hypertension, however, cannot be excluded, which warrants further investigation in prospective population-based studies.

Introduction

Hypertension is a major public health burden. In 2025, 29% of the world population is expected to have hypertension¹. Hypertension is associated with a doubled risk of cardiovascular disease and may account for 30% of all cardiovascular disease events².

The Dietary Approaches to Stop Hypertension (DASH) trial showed that a diet emphasizing increased fruit, vegetables, and low-fat dairy consumption, in addition to decreased total and saturated fat intake, substantially lowered blood pressure (BP)³. An inverse association between dairy intake and BP or hypertension has been shown in a number of cross-sectional epidemiological studies, although findings are inconclusive⁴⁻⁷. In a prospective study among 5880 university graduates in Spain (SUN cohort), low-fat dairy intake was associated with a remarkably lower incidence of hypertension after 2 y of follow-up⁸. Moore et al.⁹ observed in 95 children that diets rich in dairy products, apart from fruits and vegetables, had beneficial effects on BP change during childhood. Dairy intake, however, was not associated with BP change in the S.U.V.I.M.A.X cohort in over 2000 French adults⁴.

The amount and type of dairy foods that are consumed in different populations varies considerably, which may partly explain the inconclusive findings of previous studies. In the Netherlands, habitual dairy intake varies widely and a large variety of dairy products is consumed. We investigated whether dairy consumption, including specific dairy food groups, was associated with BP and risk of hypertension in a Dutch population-based cohort of 21,553 participants aged 20-65 y.

Methods

Study design

Data were used from the Monitoring Project on Risk Factors for Chronic Diseases (MORGEN-project), a population-based study of 23,105 men and women aged 20-65 y who were examined between 1993-1997 from 3 Dutch towns (i.e. Amsterdam, Doetinchem and Maastricht)¹⁰. We excluded 455 participants with missing dietary or BP data and 1097 participants who used antihypertensive medication, leaving 21,553 men and women for the analysis of dairy intake and BP.

Participants from Doetinchem (n = 6579) were invited for follow-up examination (including BP) in 1998-2002, and 75% (n = 4917) responded¹¹. Participants with hypertension at baseline (n = 1384) or no information on hypertension during follow-up (n = 79) were excluded, leaving 3454 participants for the analysis of dairy and incident hypertension. The study was approved by the Medical Ethics Committee of the Organization for Applied Scientific Research-Zeist, the Netherlands.

Dietary variables and exposure categories

Dietary intake was assessed using a validated, semiquantitative FFQ that inquired about intake of 178 foods and beverages during the past year¹². Total energy and nutrient intakes were calculated using a computerized Dutch Food Composition Table¹³. The reproducibility for milk and milk products after 6 and 12 mo was 0.85 and 0.73 for men and 0.75 and 0.78 for women, and the reproducibility for cheese was 0.77 and 0.71 for men and 0.67 and 0.70 for women, respectively. The validity of the FFQ was assessed against 12 monthly 24-h recalls over a 1-y period. Spearman correlations were good for milk and milk products ($r = 0.69$ for men, $r = 0.77$ for women) and moderate for cheese ($r = 0.56$ and $r = 0.32$, respectively).

Total dairy included all dairy foods except butter and ice cream. Milk and milk products included all kinds of milk, yogurt, coffee creamers, curd, pudding, porridge, custard, and whipping cream. Cheese included all types of cheese, as well as cheese spreads and cheese that was consumed during dinner or as a snack. Low-fat dairy was defined as milk and milk products with a fat concentration <2.0 g/100 g or cheese with a fat concentration <20 g/100 g. High-fat dairy was defined as milk and milk products with a fat concentration >3.5 g/100 g or cheese products with a fat concentration >20 g/100 g. Fermented dairy comprised buttermilk, yogurts, and cheese.

Outcome measurements

BP was measured with a random-zero sphygmomanometer in sitting position by trained technicians. Systolic BP was recorded at the appearance of sounds (first-phase Korotkoff) and diastolic BP at the disappearance of sounds (5th-phase Korotkoff), followed by a heart rate count for 30 s. BP measurement was repeated and values were averaged. During physical examination, regular audits were performed to check adherence to the BP measuring protocol (e.g. resting time, adequate cuff size). BP data were checked for outliers and errors before data analysis. Hypertension was defined as systolic BP ≥ 140 mm Hg or diastolic BP ≥ 90 mm Hg or use of antihypertensive medication.

Collection of risk factor data

Height was measured to the nearest 0.5 cm and weight to the nearest 0.1 kg with participants wearing light indoor clothing without shoes. BMI was calculated as weight (kg) divided by height squared (m^2) and overweight was defined as BMI ≥ 25 kg/ m^2 . Participants completed a mailed questionnaire on lifestyle factors, history of major diseases, medication use, and any prescribed diets. Questions about physical activity were added in 1994 and completed by 16,642 participants (77% of the cohort). These questionnaire data were used to create variables on alcohol intake (6 categories), smoking status (current cigarette smoking, yes/no), physical activity (h/wk), and socioeconomic status (5 categories).

Statistical analysis

Data analysis was performed using SAS version 9.1 (SAS Institute). Intakes of total dairy, dairy groups and dairy products were first adjusted for total energy intake according to the residual method²⁴. Participant characteristics in quintiles of energy-adjusted total dairy consumption are presented as means with SD or percentages. Mean BP with 95% CI were obtained in energy-adjusted dairy quintiles using a general linear model, with adjustment for age, sex, total energy intake (continuous), BMI (continuous), current cigarette smoking (yes/no), socioeconomic status (5 categories), alcohol consumption (0, 0-1, 1-2, 2-4, 4-8, >8 glasses/d), and intake of fruit, vegetables, fish, meat, bread, coffee, and tea, all as continuous variables. Additional adjustment for physical activity could only be performed in a subgroup of 16,642 participants for whom these data were available.

A number of additional predefined subgroup analyses were performed, i.e. by sex, age (<50 vs \geq 50 y), BMI (<25 vs \geq 25 kg/m²) and baseline BP (<140/90 vs \geq 140/90 mm Hg). Most of our participants appeared to be regular dairy consumers and habitual intake in our cohort was relatively high. Because the impact of dairy on BP may be more pronounced in populations with a low habitual intake of dairy, we decided to do a post hoc analysis in which we further explored the association of total and low-fat dairy with BP at low intakes. Within the bottom quintile (4310 participants), a fully adjusted linear regression coefficient for BP was obtained per 50-g increase in dairy intake. The results of the low-dairy group were then compared with the total study population.

Prospective analysis on incidence of hypertension was based on a smaller sample size (n = 3454) and energy-adjusted dairy intake was therefore divided into quartiles. Logistic regression models were used to obtain odds ratios (OR) with 95% CI for incident hypertension in categories of dairy intake, with adjustment for age and sex (model 1). Analyses were repeated using an extended multivariate model (model 2) that included age, sex, total energy intake (continuous), BMI (continuous), current cigarette smoking (yes/no), socioeconomic status (5 categories), and alcohol consumption (0, 0-1, 1-2, 2-4, 4-8, >8 glasses/d). Further adjustment was made for intake of fruit, vegetables, fish, meat, bread, coffee, and tea, all as continuous variables (model 3).

To obtain a P-value for trend across categories of dairy intake, median values of categories were assigned to individuals and entered continuously into the multivariate models. Two-sided P-values <0.05 were considered significant.

Table 2.1 Characteristics of 21,553 Dutch adults, by quintiles of energy-adjusted total dairy intake¹

	Quintiles of energy-adjusted total dairy intake				
	1	2	3	4	5
<i>n</i>	4310	4311	4311	4311	4310
Median intake, g/d	110	245	363	512	765
Sex, % male	65	43	39	39	40
Age, y	42.3 ± 10.6	42.3 ± 10.8	42.3 ± 11.2	42.5 ± 11.4	41.5 ± 11.7
Systolic blood pressure, mm Hg	121.7 ± 15.5	118.5 ± 15.6	119.4 ± 15.8	120.0 ± 16.1	119.8 ± 15.6
Diastolic blood pressure, mm Hg	77.7 ± 10.3	76.0 ± 10.5	75.8 ± 10.3	75.6 ± 10.6	75.5 ± 10.3
Hypertension ² , %	16.7	14.8	13.8	14.9	14.2
Body mass index, kg/m ²	25.1 ± 4.0	24.9 ± 3.9	24.8 ± 3.8	24.9 ± 3.8	25.0 ± 3.9
Overweight (BMI ≥ 25 kg/m ²), %	46.1	43.6	41.8	43.7	44.2
Self-reported history of myocardial infarction, %	1.1	0.7	0.8	0.9	0.8
Self-reported diabetes, %	0.7	0.7	0.9	1.5	1.5
High socioeconomic status, %	20	23	26	25	26
Physical activity by intensity ³ , h/wk					
Low (MET <4)	26.6 ± 19.0	27.6 ± 19.1	27.1 ± 18.8	27.9 ± 18.8	28.3 ± 18.5
Medium/high (MET ≥4)	9.6 ± 14.1	7.8 ± 10.8	8.4 ± 10.7	8.6 ± 10.7	9.1 ± 11.2
Current alcohol consumer, %	88	85	86	85	84
Current smoker, %	44	38	36	33	32
Dietary intake					
Total energy, MJ/d	10.8 ± 3.1	8.8 ± 2.8	8.8 ± 2.6	9.1 ± 2.6	10.0 ± 3.1
Total dairy, g/d	140 ± 94	217 ± 110	337 ± 108	498 ± 115	860 ± 312
Milk & milk products	106.1 ± 86.8	183.0 ± 104.4	301.7 ± 102.5	462.9 ± 109.7	822.1 ± 307.6
Cheese	34.0 ± 32.0	34.4 ± 29.2	34.9 ± 28.1	35.5 ± 27.9	37.5 ± 31.0
Low-fat dairy	38.9 ± 46.0	86.3 ± 70.5	163.3 ± 83.6	277.6 ± 105.9	535.6 ± 259.9
Fermented dairy	63.7 ± 50.7	92.8 ± 67.9	133.8 ± 94.5	181.8 ± 133.6	294.8 ± 264.5
Fruit, g/d	135 ± 137	141 ± 122	152 ± 120	163 ± 117	173 ± 130
Vegetables, g/d	123 ± 54	118 ± 50	122 ± 52	123 ± 50	127 ± 53
Fish, g/d	8.3 ± 9.8	8.8 ± 10.0	8.5 ± 8.7	8.9 ± 10.4	9.3 ± 10.4
Meat, g/d	156.2 ± 72.1	116.5 ± 57.7	108.7 ± 56.1	108.4 ± 53.4	106.8 ± 58.6

Quintiles of energy-adjusted total dairy intake

	1	2	3	4	5
Bread, g/d	189.2 ± 89.0	149.3 ± 72.1	146.4 ± 68.2	146.7 ± 66.7	148.0 ± 74.9
Coffee, mL/d	510.4 ± 390.1	448.0 ± 342.4	430.3 ± 317.3	422.8 ± 303.7	407.8 ± 317.7
Tea, mL/d	181.3 ± 241.1	194.2 ± 230.7	196.5 ± 225.4	199.3 ± 214.9	200.2 ± 220.0
Total fat, g/d	106.1 ± 36.4	85.8 ± 31.1	84.3 ± 30.0	86.0 ± 29.2	90.9 ± 33.0
Saturated fat, g/d	41.8 ± 15.6	34.6 ± 13.3	34.7 ± 12.6	36.0 ± 12.7	39.5 ± 14.9
Total protein, g/d	86.5 ± 24.5	74.7 ± 23.1	77.1 ± 21.8	83.1 ± 21.7	97.0 ± 27.8
Total calcium, mg/d	788 ± 320	837 ± 313	982 ± 300	1181 ± 306	1637 ± 501

¹ Values are means ± SD or %

² Hypertension is defined as systolic BP ≥140 mm Hg or diastolic BP ≥90 mm Hg (participants using antihypertensive medication were excluded).

³ Data on physical activity was available in 16,642 participants; n = 3283 for the first quintile, n = 3303 for the 2nd quintile, n = 3349 for the 3rd quintile; n = 3319 for the 4th quintile, and n = 3388 for the 5th quintile.

Results

Population characteristics

Participant characteristics according to energy-adjusted dairy intake are shown (**Table 2.1**). Mean BP was 120.1 ± 15.7 mm Hg systolic and 76.1 ± 10.4 mm Hg diastolic and 15% of the population had elevated BP (i.e. $\geq 140/90$ mm Hg). Dairy intake was associated with fruit intake (positively), coffee intake (inversely), and nonsmoking. In the lowest dairy category, that included more men, intakes of total energy, meat, bread, and total and saturated fat were higher than in other categories.

Dairy consumption

The median energy-adjusted total dairy intake of the study population was 344 g/d, ranging from 110 g/d (i.e. <1 serving/d) in the lowest quintile to 765 g/d (i.e. >5 servings/d) in the highest quintile. Dairy food items that contributed to total dairy intake are presented (**Supplemental table 2.1**). The Spearman correlation coefficient was 0.91 ($P < 0.0001$) for the relation between total dairy and low-fat dairy and 0.41 ($P < 0.0001$) for the relation between low-fat and high-fat dairy.

Dairy intake and BP

BP showed little variation across quintiles of total dairy, low-fat dairy, and high-fat dairy (**Table 2.2**). For other dairy groups and products, BP was also similar across quintiles of intake (**Supplemental table 2.2**). Excluding desserts from the milk products category did not change the findings. Moreover, additional adjustment for complementary dairy food groups (e.g. low-fat dairy as a covariate when studying high-fat dairy) or saturated fat did not materially change the results. The results did also not change when we reanalyzed the data after exclusion of participants with self-reported myocardial infarction or diabetes and additional adjustment for prescribed diet. Furthermore, additional inclusion of physical activity in the model, using data of a subgroup of 16,642 participants in whom this was assessed, did not change the results (data not shown).

Subgroup analyses showed that the association between low-fat dairy and BP did not vary among strata of age, sex, BMI, or BP (data not shown). When we examined the relation between low-fat dairy and BP continuously in the total cohort, the fully adjusted β (95% CI) per 50-g increase was 0.05 mm Hg (0.02, 0.09) for systolic BP and -0.04 mm Hg ($-0.06, -0.02$) for diastolic BP. In the subgroup ($n = 4310$) with a low habitual intake of dairy, these β were 0.19 mm Hg ($-0.13, 0.50$) and -0.09 mm Hg ($-0.30, 0.12$) per 50 g, respectively.

Table 2.2 BP in 21,553 Dutch adults by type of dairy intake¹

	Quintiles of energy-adjusted dairy intake					P-trend
	1	2	3	4	5	
<i>n</i>	4310	4311	4311	4311	4310	
Total dairy						
Median intake, g/d	110	245	363	512	765	
SBP ² , mm Hg	119.7 (119.3, 120.2)	119.6 (119.2, 120.0)	120.0 (119.6, 120.4)	120.5 (120.1, 120.9)	120.6 (120.2, 121.0)	<0.0001
DBP ³ , mm Hg	76.7 (76.4, 76.9)	76.1 (75.8, 76.4)	76.1 (75.9, 76.4)	76.0 (75.7, 76.2)	75.9 (75.6, 76.1)	<0.0001
High-fat dairy						
Median intake, g/d	27	63	88	116	188	
SBP, mm Hg	119.8 (119.4, 120.3)	120.1 (119.7, 120.5)	120.3 (119.9, 120.8)	120.1 (119.7, 120.5)	120.0 (119.6, 120.4)	0.82
DBP, mm Hg	76.4 (76.1, 76.7)	76.3 (76.0, 76.6)	76.4 (76.1, 76.7)	75.9 (75.6, 76.2)	75.6 (75.4, 75.9)	<0.0001
Low-fat dairy						
Median intake, g/d	21	89	174	294	507	
SBP, mm Hg	119.6 (119.2, 120.1)	119.8 (119.4, 120.3)	119.9 (119.5, 120.3)	120.4 (120.0, 120.8)	120.6 (120.2, 121.0)	<0.0001
DBP, mm Hg	76.5 (76.2, 76.8)	76.2 (76.0, 76.5)	76.2 (75.9, 76.5)	75.8 (75.5, 76.1)	76.0 (75.7, 76.3)	<0.01

¹ Values are mean BP and 95% CI, adjusted for age (y), sex, total energy intake (MJ/d), socioeconomic status (5 categories), BMI (kg/m²), smoking (yes/no), and daily intake of alcohol (6 categories), fruit (g), vegetables (g), fish (g), meat (g), bread (g), coffee (mL) and tea (mL).

² SBP, Systolic BP.

³ DBP, Diastolic BP.

Dairy intake and incidence of hypertension

The subcohort that participated in the follow-up round was 49.5 ± 9.6 y and comprised 45 % men. BP in these participants increased by 5.7 ± 12.7 mm Hg systolic and 3.1 ± 9.6 mm Hg diastolic during a median follow-up period of 5 y and 713 (20.6%) new cases of hypertension were identified.

After adjustment for potential confounders, total dairy, low-fat dairy and high-fat dairy were not significantly associated with risk of hypertension (P-trend 0.60, 0.24 and 0.11 respectively) (**Table 2.3**). Also, other dairy groups and dairy products were not clearly related to risk of hypertension (**Supplemental table 2.3**).

After including physical activity in the multivariate model ($n = 2768$), OR (95% CI) in the consecutive quartiles of low-fat dairy were 1.00, 0.83 (0.63, 1.10), 0.81 (0.61, 1.07), and 0.85 (0.64, 1.13) respectively (P-trend: 0.36). The association between low-fat dairy and hypertension risk did not vary by sex (**Table 2.3**) or age (data not shown). After stratification by BMI (<25 vs ≥ 25 kg/m²), OR in the 2nd to the 4th quartiles of low-fat dairy intake were 0.95 (0.68, 1.32), 0.97 (0.70, 1.36), and 0.86 (0.61, 1.21; P-trend: 0.42) for participants who were overweight and 0.60 (0.42, 0.87), 0.63 (0.43, 0.91), and 0.76 (0.52, 1.11; P-trend: 0.34), respectively for participants who were not overweight.

Discussion

In this cohort of 21,553 Dutch adults, overall dairy intake or intake of specific dairy groups or dairy products was not clearly related to BP. Prospective analysis in 3454 participants showed no consistent association of dairy intake with 5-y risk of hypertension, with a possible exception for low-fat dairy that was nonsignificantly related to a lower risk of hypertension (P-trend: 0.24).

This study has several strengths and limitations. Our Dutch study is based in a country with a naturally high intake of dairy. It comprized regular consumers of a variety of dairy products, enabling us to examine a broad range of several types of dairy products in relation to BP. Total dairy intake ranged from <1 to >5 servings/d. The large sample size allowed more detailed examination of BP in participants who consumed a low amount of dairy (i.e. <185 g/d), but associations with BP were not found within this low range of intake.

A major concern in epidemiological studies is residual confounding. Data on physical activity, an important BP determinant, was available for only 77% of our participants. Repeating multivariate analysis with additional adjustment for physical activity in this subgroup did not influence the BP associations and we think it is unlikely that incomplete physical activity data has impacted our findings. Dairy intake was assessed

Table 2.3 Association of dairy intake with incident hypertension in 3454 Dutch adults¹

	Quartiles of energy-adjusted dairy intake				P-trend
	1	2	3	4	
<i>n</i>	863	864	864	863	
Total dairy					
Median intake, g/d	206	359	510	757	
Incident cases, <i>n</i>	185	189	162	177	
OR, model 1 ²	1.00	1.01 (0.80, 1.27)	0.85 (0.67, 1.08)	0.98 (0.77, 1.24)	0.66
OR, model 2 ³	1.00	1.06 (0.83, 1.35)	0.93 (0.72, 1.20)	1.07 (0.83, 1.37)	0.77
OR, model 3 ⁴	1.00	1.08 (0.84, 1.38)	0.95 (0.73, 1.22)	1.11 (0.85, 1.44)	0.60
Men (<i>n</i> = 1549)	1.00	1.01 (0.73, 1.41)	0.78 (0.54, 1.12)	1.12 (0.78, 1.62)	0.81
Women (<i>n</i> = 1905)	1.00	1.15 (0.78, 1.69)	1.11 (0.76, 1.62)	1.12 (0.76, 1.66)	0.70
Low-fat dairy					
Median intake, g/d	59	167	296	500	
Incident cases, <i>n</i>	207	167	171	168	
OR, model 1	1.00	0.77 (0.61, 0.98)	0.79 (0.62, 0.99)	0.80 (0.63, 1.01)	0.13
OR, model 2	1.00	0.78 (0.61, 0.99)	0.80 (0.63, 1.02)	0.81 (0.64, 1.04)	0.20
OR, model 3	1.00	0.78 (0.61, 1.00)	0.81 (0.63, 1.03)	0.82 (0.64, 1.06)	0.24
Men (<i>n</i> = 1549)	1.00	0.75 (0.54, 1.04)	0.73 (0.52, 1.05)	0.86 (0.60, 1.23)	0.43
Women (<i>n</i> = 1905)	1.00	0.81 (0.56, 1.18)	0.88 (0.61, 1.25)	0.82 (0.57, 1.18)	0.43
High-fat dairy					
Median intake, g/d	43	77	106	166	
Incident cases, <i>n</i>	178	176	172	187	
OR, model 1	1.00	0.94 (0.74, 1.20)	0.92 (0.72, 1.17)	1.05 (0.83, 1.34)	0.58
OR, model 2	1.00	0.91 (0.70, 1.17)	0.91 (0.70, 1.17)	1.13 (0.88, 1.45)	0.20
OR, model 3	1.00	0.93 (0.72, 1.19)	0.93 (0.72, 1.21)	1.19 (0.92, 1.54)	0.11
Men (<i>n</i> = 1549)	1.00	0.90 (0.64, 1.26)	0.96 (0.67, 1.39)	1.45 (1.03, 2.06)	0.03
Women (<i>n</i> = 1905)	1.00	0.87 (0.59, 1.28)	0.79 (0.54, 1.18)	1.05 (1.03, 1.06)	0.74

¹ Values are OR with 95% CI.

² Adjusted for age and sex.

³ Adjusted for age, sex, total energy intake (MJ/day), socioeconomic status (5 categories), BMI (kg/m²), smoking (yes/no), and alcohol intake (6 categories).

⁴ Adjusted for age, sex, total energy intake (MJ/day), socioeconomic status (5 categories), BMI (kg/m²), smoking (yes/no), and alcohol intake (6 categories) and daily intake of fruit (g), vegetables (g), fish (g), meat (g), bread (g), coffee (mL), and tea (mL).

by self-report, which may have led to misclassification. Assessment of dairy intake in our study was validated against 12 monthly repeated 24-h recalls. Milk and milk products were highly correlated ($r = 0.7-0.8$), indicating that participants could adequately be ranked according to milk intake¹². The validity for cheese, however, was much lower ($r = 0.4-0.5$), which may have diluted the cheese-BP associations. It cannot, however, explain our overall null result, because cheese contributed only 8.6% to total dairy intake.

Scientific evidence for an effect of dairy on BP or hypertension is inconsistent. Our data are in line with findings from the French SU.VI.MAX study among 4652 participants aged 35-63 y, in which overall dairy was not associated with BP or change⁴. Ruidavets et al.⁶, however, reported that dairy products (range, 93 – 335 g/d) and dietary calcium were significantly and independently associated with lower systolic BP (7 mm Hg for total dairy and 4 mm Hg for calcium) in 912 French men aged 45-64 y. Also, Djoussé et al.⁵ found that dairy intake (range, 0.4–3.1 servings/d) was associated with a 38% lower prevalence of hypertension, independent of calcium, in the NHLBI Family Heart Study. Dairy intake in our study was higher (344 g/d, ~2.5 servings), which may explain differences in results. It may be possible that the impact of dairy intake on BP is more pronounced in persons with a low habitual intake of dairy. However, repeating our analyses within the low end of the distribution of dairy intake (0-185 g/d) yielded similar conclusions. In a previous cross-sectional study of 2064 Dutch men and women aged 50-75 y⁷, intake of dairy, in particular dairy desserts, milk, and yogurt, was inversely associated with BP. Apart from the somewhat higher dairy intake (4 servings/d), participants in that study were older than in our cohort, which may partly account for the discrepancy in findings. When reanalyzing our data in participants aged ≥ 50 y, however, we still did not find an association of dairy with BP. Snijder et al.⁷ found an association of high-fat rather than low-fat dairy with BP, in contrast to our findings for incident hypertension. Alonso et al.⁸, on the other hand, showed that low-fat dairy (mean intake of 140 g/d), but not high-fat dairy, was associated with a remarkable 54% reduced risk of hypertension during 2 y of follow-up of 5880 Spanish university graduates. A potential beneficial effect of low-fat dairy is consistent with the DASH trial in which BP reductions were more pronounced when low-fat dairy (among other dietary changes) was added to a fruits-and-vegetables diet³. In the Coronary Artery Risk Development in Young Adults (CARDIA) study, both reduced-fat and high-fat dairy products were associated with a 21% reduced odds of 10-y incidence of elevated BP in 923 young overweight adults, but not in 2234 normal weight participants¹⁵. Overall dairy intake was not related to 15-y incidence of elevated BP in the CARDIA study, whereas milk and dairy desserts had an inverse association¹⁶.

It is yet unclear which specific dairy foods or components could be responsible for a beneficial effect on BP. Dairy is an important source of protein and minerals (e.g. calcium, potassium, magnesium) which may lower BP¹⁷⁻²¹. Because vitamin D is critical for calcium absorption and homeostasis²², vitamin D may also play a role in the prevention of hypertension²³. Wang et al.²⁴ showed that intakes of low-fat dairy products, calcium, and vitamin D were each inversely associated with risk of hypertension in 28,886 middle-aged and older women. In the Netherlands, however, milk products have not yet been fortified with vitamin D. It can be speculated that low-fat dairy may be more beneficial to BP than high-fat dairy because of its reduced concentration of

saturated and natural *trans* fatty acids, which may mitigate the beneficial effects of other dairy components.

Our findings suggest that dairy intake has little effect on population BP in a generally healthy population of young and middle-aged Dutch adults. We found some evidence for a beneficial effect of low-fat dairy on risk of hypertension, although not significant, which needs to be confirmed in other population-based cohort studies.

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Conflict of interest

FJ Kok is a member of the Communication and Scientific Advisory Board of the Global Dairy Platform (www.globaldairyplatform.com/Global-Dairy-Platform/Header/About-GDP). MF Engberink, JM Geleijnse, N de Jong, HA Smit and WMM Verschuren have no conflict of interest.

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Supplemental table 2.1
Dairy food items consumed by 21,553 Dutch adults

	Median intake (IQR), g/d ¹	Contribution to total dairy intake, %
Total dairy	344 (191,558)	
Milk & milk products	308 (156,523)	91.4
Raw milk ²	11 (2,25)	4.2
High-fat milk ²	14 (3,32)	8.8
Semi-skimmed milk ³	40 (5,139)	24.8
Skimmed milk ³	12 (2,27)	5.9
Buttermilk ^{3,4}	0 (0,20)	11.6
Chocolate milk ³	15 (2,33)	7.2
Milk in coffee	2 (0,6)	1.7
High-fat yogurt ^{2,4}	0 (0,1)	0.9
Semi-skimmed yogurt ⁴	7 (2,22)	3.8
Low-fat yogurt ^{3,4}	8 (3,25)	5.0
Yogurt-based drinks ^{3,4}	0 (0,7)	5.4
Coffee creamers ²	6 (0,21)	3.2
Curd ^{3,4}	5 (2,11)	2.1
Pudding	6 (2,13)	2.4
Porridge, custard	8 (2,23)	3.8
Whipping cream ²	2 (1,3)	0.6
Cheese ⁴	29 (15,46)	8.6
High-fat ²	24 (12,41)	7.6
Reduced fat ³	2 (1,4)	1.0

¹ Data presented as median values (Inter Quartile Range) because of skewed distribution.

² High-fat dairy products.

³ Low-fat dairy products.

⁴ Fermented dairy products.

Supplemental table 2.2 Blood pressure in 21,553 Dutch adults by type of dairy intake¹

	Quintiles of energy-adjusted dairy intake					P-trend
	1	2	3	4	5	
<i>n</i>	4310	4311	4311	4311	4310	
Fermented dairy						
Median intake, g/d						
SBP ² , mm Hg	119.8 (119.3, 120.2)	120.1 (119.7, 120.6)	120.3 (119.9, 120.7)	120.2 (119.8, 120.6)	120.0 (119.6, 120.4)	0.93
DBP ³ , mm Hg	76.4 (76.1, 76.7)	76.2 (75.9, 76.4)	76.3 (76.0, 76.5)	75.9 (75.7, 76.2)	75.9 (75.6, 76.2)	0.01
Milk products						
Median intake, g/d						
SBP, mm Hg	119.6 (119.2, 120.1)	119.6 (119.2, 120.1)	120.0 (119.5, 120.4)	120.5 (120.1, 120.9)	120.7 (120.2, 121.1)	<0.0001
DBP, mm Hg	76.6 (76.3, 76.9)	76.1 (75.8, 76.4)	76.1 (75.8, 76.4)	76.0 (75.7, 76.3)	75.9 (75.6, 76.2)	<0.01
Cheese						
Median intake, g/d						
SBP, mm Hg	120.1 (119.7, 120.6)	120.3 (119.9, 120.7)	119.9 (119.5, 120.3)	120.5 (120.1, 120.9)	119.6 (119.1, 120.0)	0.08
DBP, mm Hg	76.3 (76.0, 76.6)	76.2 (75.9, 76.5)	76.0 (75.7, 76.3)	76.3 (76.0, 76.6)	76.0 (75.7, 76.3)	0.27
Yogurt						
Median intake, g/d						
SBP, mm Hg	120.0 (119.6, 120.5)	119.9 (119.5, 120.4)	119.9 (119.5, 120.3)	120.4 (120.0, 120.8)	120.1 (119.7, 120.6)	0.46
DBP, mm Hg	76.4 (76.1, 76.7)	76.2 (75.9, 76.5)	75.8 (75.5, 76.1)	76.4 (76.1, 76.6)	75.9 (75.6, 76.2)	0.06

¹ Values are means (95% CI) adjusted for age (y), sex, total energy intake (MJ/d), socio-economic status (5 categories), BMI (kg/m²), smoking (yes/no), and daily intake of alcohol (6 categories), fruit (g), vegetables (g), fish (g), meat (g), bread (g), coffee (g), tea (mL).

² SBP, systolic blood pressure.

³ DBP, diastolic blood pressure.

Supplemental table 2.3 Odds ratios (OR) for incident hypertension after 5 years of follow-up in 3454 Dutch adults by type of dairy intake¹

	Quartiles of energy-adjusted dairy intake				P-trend
	1	2	3	4	
<i>n</i>	863	864	864	863	
Fermented dairy					
Median intake, g/d	54	105	172	358	
Incident cases, n	186	179	173	175	
Multivariate OR	1.00	1.01 (0.79, 1.30)	1.00 (0.78, 1.29)	1.00 (0.77, 1.29)	0.93
Milk & milk products					
Median intake, g/d	169	321	479	719	
Incident cases, n	191	185	157	180	
Multivariate OR	1.00	1.00 (0.78, 1.27)	0.86 (0.66, 1.11)	1.09 (0.84, 1.42)	0.63
Cheese					
Median intake, g/d	12	26	40	62	
Incident cases, n	153	210	181	169	
Multivariate OR	1.00	1.45 (1.13, 1.87)	1.19 (0.92, 1.55)	1.17 (0.90, 1.52)	0.77
Yogurt					
Median intake, g/d	12	39	70	122	
Incident cases, n	205	176	159	173	
Multivariate OR	1.00	0.88 (0.69, 1.12)	0.83 (0.65, 1.07)	0.88 (0.69, 1.12)	0.36

¹ OR (95% CI) adjusted for age (y), sex, total energy intake (MJ/d), socio-economic status (5 categories), BMI (kg/m²), smoking (yes/no), and daily intake of alcohol (6 categories), fruit (g), vegetables (g), fish (g), meat (g), bread (g), coffee (mL) and tea (mL).



Chapter 3

Inverse association between dairy intake and hypertension:
The Rotterdam Study

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Abstract

Background: Little is known about the effect of different types of dairy food products on the development of hypertension.

Methods: We examined the relation between dairy intake and incident hypertension in 2245 participants of the Rotterdam Study aged 55 y and over with complete dietary and blood pressure data, who were free of hypertension at baseline (1990-1993). Blood pressure was reexamined in 1993-1995 and in 1997-1999. Hazard ratios (HR, with 95%-confidence interval) for 2-y and 6-y incidence of hypertension were obtained in quartiles of energy-adjusted dairy intake, with adjustment for age, sex, BMI, smoking, educational level, and intake of alcohol, total energy and dietary factors.

Results: Risk of hypertension after 2 y of follow-up (664 incident cases) was inversely associated with dairy intake. After adjustment for confounders, HR (95% CI) were 1.00, 0.82 (0.67, 1.02), 0.67 (0.54, 0.84), and 0.76 (0.61, 0.95) in consecutive quartiles of total dairy (P-trend: 0.008). Corresponding HR for low-fat dairy were 1.00, 0.75 (0.60, 0.92), 0.77 (0.63, 0.96) and 0.69 (0.56, 0.86, P-trend: 0.003). Analysis of specific types of dairy showed an inverse association with milk and milk products (P-trend: 0.07) and no association with high-fat dairy or cheese ($p > 0.6$). After 6 y of follow-up (984 incident cases), the associations with hypertension were attenuated to risk reductions around 20% for both total and low-fat dairy between the extreme quartiles of intake (P-trend: 0.07 and 0.09, respectively).

Conclusion: Intake of low-fat dairy may contribute to the prevention of hypertension at older age.

Introduction

Diet and lifestyle play an important role in blood pressure control. The DASH trial showed that a diet rich in fruit, vegetables and low-fat dairy products can substantially reduce blood pressure¹. Remarkably, this diet showed a more pronounced effect than a diet rich in fruit and vegetables in which dairy consumption was low. This drew the attention to the possible role of (low-fat) dairy foods in the prevention of hypertension. Although the underlying mechanism remains to be established, it has been linked to protein², bioactive peptides³ and minerals such as calcium, potassium or magnesium⁴⁻⁸.

The association between dairy intake and blood pressure or hypertension has been examined in several cross-sectional⁹⁻¹¹ and prospective studies¹²⁻¹⁵ with results generally suggesting an inverse association. The NHLBI Family Heart Study showed a 36% lower prevalence of hypertension in American adults with a high intake of dairy products, independent of calcium intake⁹. A cross-sectional study among 912 middle-aged French men showed that dairy products and dietary calcium were both significantly and independently associated with lower levels of systolic blood pressure (7 mm Hg for total dairy and 4 mm Hg for calcium)¹⁰. Beneficial effects on childhood blood pressure have been reported in the Framingham Children's study, for low-fat as well as high-fat dairy products¹⁶. The CARDIA study among young adults, reported a lower incidence of hypertension in overweight subjects with dairy consumption in the highest quartile¹³. The SUN cohort study among Spanish university graduates prospectively showed that low-fat dairy intake, but not whole-fat dairy, was associated with 54% lower risk of incident hypertension in the upper quartile¹². Wang et al. reported that low-fat dairy products, calcium, and vitamin D were each inversely associated with risk of hypertension in middle-aged and older women¹⁴.

Little is known about the effect of different types of dairy food products on the development of hypertension. In the Netherlands, a variety of dairy products is commonly consumed which allows detailed examination of the association between different types of dairy food products and hypertension over a broad range of intake. In the present study, we examined whether incidence of hypertension in older Dutch subjects was associated with intake of total dairy, high-fat dairy, low-fat dairy, fermented dairy, milk and milk products, and cheese products.

Methods

Study population

The present analysis formed part of the Rotterdam Study, a population-based cohort study on the occurrence and progression of chronic diseases and their risk factors in people aged 55 y and over¹⁷. In brief, between 1990 and mid 1993 all residents of a

suburb of Rotterdam in this age category were invited to participate and 7983 (78%) subjects responded. All participants were interviewed at home and 89% of the cohort was physically examined during 2 baseline visits at the research center. The cohort was reexamined during follow-up in 1993-1995 (79% response) and 1997-1999 (76% response). Written informed consent was obtained from all participants. The medical ethics committee of Erasmus University approved the study protocol.

Dietary assessment and exposure categories

At baseline, participants completed a checklist at home that inquired foods and drinks they had consumed at least twice a month during the preceding year as well as dietary habits, use of supplements, and prescribed diets. Next, during their visit to the research center, they underwent a standardized interview with a trained dietician based on the checklist, using a 170-item semiquantitative food frequency questionnaire. A validation study comparing this questionnaire with a 2-week food diary demonstrated reproducible and valid estimates¹⁸. These dietary data were converted to total energy intake and nutrient intake per day with the computerized Dutch Food Composition Table taking seasonal variation into account¹⁹.

For the current study, we calculated total dairy intake by summing the intake of individual dairy items except butter and ice cream. Subsequently, we defined five categories of specific types of dairy foods which are not mutually exclusive. The category 'milk and milk products' included all kind of milk, yogurt, coffee creamer, curd, pudding, porridge, custard, and whipped cream. The category 'cheese' included all kind of cheese products, i.e. soft cheese, hard cheese and cheese spreads. The category 'low-fat dairy' was defined as all milk and milk products with a fat content less than 2.0 g/100 g and all cheese products with a fat content less than 20 g/100 g. The category 'high-fat dairy' was defined as all milk and milk products with a fat content above 3.5 g/100 g and all cheese products with a fat content higher than 20 g/100 g. The category 'fermented dairy' included all fermented dairy products such as buttermilk, yogurts, curds, and cheese products (both low-fat and high-fat). The food frequency questionnaire was not specifically validated for dairy intake. However, dairy foods are rich in protein and calcium; it attributes about 25-30% to the total daily protein intake and 70% to the daily calcium intake in the Dutch situation²⁰. High correlations ($r = 0.6-0.7$) were found for these two dairy nutrients¹⁶.

Outcome measurements

Blood pressure measurements were taken at the research center at baseline and during follow-up examinations. Blood pressure was measured at the right upper arm using a random-zero sphygmomanometer after the participant had been seated for at least 5 minutes. Systolic and diastolic blood pressures were calculated as the average of two

consecutive measurements. Hypertension was defined as a systolic blood pressure ≥ 140 mm Hg or a diastolic blood pressure ≥ 90 mm Hg or the use of antihypertensive medication which was assessed at baseline and during follow-up. At the research center, a physician ascertained the indication for which the medication had been prescribed.

Collection of risk factor data

Information on current health status, medical history, medication, smoking behavior and education was obtained by a trained research assistant who used a computerized questionnaire. Participants were classified as current smokers, former smokers or never smokers. Education was defined as low (primary education), intermediate (secondary general or vocational education) and high (higher vocational education or university). Weight and height were measured during the visit to the research center and body mass index (BMI) was calculated as weight in kg divided by the square of height in meters (kg/m^2). Alcohol intake was assessed in grams of ethanol per day. Information on prevalent cardiovascular disease, defined as a history of myocardial infarction or stroke, was assessed during a home interview and verified in medical records of the general practitioner. Serum total cholesterol was measured with an automated enzymatic method in a non-fasting blood sample. Diabetes was defined as the use of antidiabetic medication or a post- or preload serum glucose level ≥ 11.1 mmol/L.

Population for analysis

Our study population consisted of 5435 participants in the Rotterdam Study for whom a completed food frequency questionnaire was available. We excluded 2912 subjects with hypertension at baseline and 22 subjects without information on hypertension status at baseline (blood pressure not measured). Additionally, we excluded 256 without information on hypertension status at both follow-up examinations since they were either lost to follow-up ($n = 128$) or died ($n = 128$) and blood pressure was not remeasured after baseline. This resulted in 2245 subjects for the present analysis. Subjects who were excluded from the analysis did not differ with respect to blood pressure and dairy intake at baseline.

Data analysis

Total dairy and intake of specific dairy food groups were adjusted for total energy intake according to the residual method²¹ and subsequently categorized into quartiles. We compared subject characteristics at baseline across quartiles of total energy-adjusted dairy intake to identify potential confounders. We used Cox proportional hazard modeling to estimate hazard ratios (HR with 95%-confidence interval) for incident hypertension in quartiles of energy-adjusted dairy intake, using the lowest quartile as the reference. For non-hypertensive participants, we computed person-time of follow-up from baseline to the end of the study period. For participants who developed

hypertension, we attributed 1 y of follow-up if hypertension was identified during the 2-y examination visit and 4 y of follow-up if hypertension was identified during the 6-y examination visit. We first calculated hazard ratios for the association between dairy intake and 2-y incidence of hypertension. The basic model included age (continuous) and sex. Subsequently, we performed a multivariate analysis (model 1) with adjustment for age, sex, total energy (continuous), BMI (continuous), smoking status (yes/no), alcohol consumption (3 categories) and educational level (3 categories). Further adjustment (model 2) was then made for intake of fruit (including fruit juices), vegetables, meat, bread, coffee, and tea (continuous). We assessed linear trends across dairy intake categories by entering the median values within quartiles into the model as a linear covariate. We performed several pre-defined stratified analyses based on results of previous studies examining the association between dairy intake and hypertension^{13, 22}. These subgroup analyses were performed in strata of sex and overweight (i.e., BMI ≥ 25 kg/m²), using model 2. Finally, we repeated all analyses for the association between dairy intake and 6-y incidence of hypertension. Data-analysis was performed using SAS software (SAS Institute) version 9.1 and reported p-values are two-sided.

Results

Baseline characteristics

Baseline characteristics of the study population in quartiles of energy-adjusted total dairy intake are shown in **Table 3.1**. A higher dairy intake was associated with a lower consumption of meat, bread, and coffee. In the lowest category of dairy intake, that included more men and current smokers, intakes of total energy, total and saturated fat, and alcohol were higher than in other categories.

Dairy consumption

The median energy-adjusted total dairy intake of the study population was 396 g/d, ranging from 164 g/d (i.e. ~1 serving/d) in the lowest quartile to 691 g/d (i.e. ~4.5 servings/d) in the highest quartile. Low-fat milk (31%), buttermilk (17%), high-fat milk (10%), low-fat yogurt (9%) and Gouda cheese (7%) made the largest contribution to the total dairy intake. Total dairy was highly correlated with low-fat dairy (Spearman correlation, $r = 0.77$, $p < 0.0001$).

Dairy intake and 2-year incidence of hypertension

During 2 y of follow-up, we identified 664 incident cases of hypertension. After adjusting for age, sex, BMI, educational level, smoking, total energy intake, alcohol consumption and several dietary factors, hazard ratios (95% CI) across increasing quartiles of total dairy were 1.00, 0.82 (0.67, 1.02), 0.67 (0.54, 0.84), and 0.76 (0.61, 0.95, P-trend 0.008, **Table 3.2**). Each serving/d (defined as 150 mL) increase in total dairy intake was

Table 3.1 Baseline characteristics of 2245 Dutch adults (≥ 55 y) across quartiles of energy-adjusted total dairy intake

	Quartiles of energy-adjusted total dairy intake			
	1	2	3	4
<i>n</i>	561	561	562	561
Median dairy intake ¹ , g/d	164	325	472	691
Age, y	64.9 \pm 6.7	65.3 \pm 6.5	65.7 \pm 7.1	65.2 \pm 7.5
Gender, % male	56	43	37	35
Body mass index, kg/m ²	25.3 \pm 3.2	25.8 \pm 3.4	25.9 \pm 3.4	25.7 \pm 3.4
Overweight (≥ 25 kg/m ²), %	48	58	56	56
Educational level, % high	12	11	13	11
Current smoker, %	31	23	24	24
Alcohol, % abstainers	16	17	17	21
Intake among users, g/d ²	13.0 (3.4, 27.3)	6.9 (1.6, 20.0)	6.2 (1.5, 14.8)	4.7 (0.8, 13.2)
Systolic BP, mm Hg	121.8 \pm 11.8	121.7 \pm 11.9	122.6 \pm 11.7	121.3 \pm 12.3
Diastolic BP, mm Hg	68.7 \pm 8.3	68.6 \pm 8.7	68.8 \pm 8.2	67.9 \pm 9.0
History of self-reported DM, %	4.6	4.8	6.3	6.6
History of CVD, %	11.9	10.2	10.0	8.7
Total cholesterol, mmol/L	6.6 \pm 1.3	6.6 \pm 1.1	6.6 \pm 1.2	6.6 \pm 1.1
HDL cholesterol, mmol/L	1.36 \pm 0.36	1.36 \pm 0.36	1.37 \pm 0.35	1.37 \pm 0.35
Dietary intakes				
Total dairy ³ , g/d	170 \pm 90	327 \pm 82	471 \pm 83	777 \pm 251
Low-fat dairy ³ , g/d	77 \pm 79	197 \pm 105	315 \pm 135	555 \pm 289
High-fat dairy ⁴ , g/d	93 \pm 65	130 \pm 102	156 \pm 144	222 \pm 258
Fermented dairy ⁵ , g/d	78 \pm 62	148 \pm 97	206 \pm 134	295 \pm 239
Milk & milk products ⁶ , g/d	134 \pm 87	291 \pm 79	435 \pm 79	737 \pm 253
Cheese ⁷ , g/d	36 \pm 24	36 \pm 23	36 \pm 21	39 \pm 27
Meat, g/d	126 \pm 54	111 \pm 45	102 \pm 45	102 \pm 47
Fish, g/d	14.5 \pm 18.2	16.2 \pm 18.8	15.1 \pm 16.8	15.4 \pm 18.1
Bread, g/d	149 \pm 61	139 \pm 54	136 \pm 56	133 \pm 54
Vegetables, g/d	378 \pm 152	359 \pm 117	344 \pm 127	346 \pm 121
Fruit, g/d	211 \pm 147	232 \pm 131	238 \pm 121	243 \pm 132
Coffee, mL/d	538 \pm 277	509 \pm 236	504 \pm 231	492 \pm 247
Tea, mL/d	361 \pm 277	359 \pm 247	366 \pm 259	356 \pm 253
Total energy, MJ/d	8.9 \pm 2.3	8.3 \pm 2.0	8.2 \pm 2.0	8.6 \pm 2.2
Total fat, g/d	90.0 \pm 31.5	81.4 \pm 25.4	79.0 \pm 26.1	82.0 \pm 27.7
Saturated fat, g/d	34.7 \pm 13.1	31.6 \pm 10.5	31.2 \pm 11.2	33.2 \pm 13.1
Total protein, g/d	78.1 \pm 18.7	79.1 \pm 17.4	81.2 \pm 18.0	92.7 \pm 20.5
Total calcium, mg/d	809 \pm 256	1001 \pm 242	1170 \pm 240	1569 \pm 399

Data are presented as mean \pm SD or %.

BMI, Body Mass Index; BP, Blood pressure; CVD, Cardiovascular diseases; DM, Diabetes Mellitus.

¹ Included all dairy products except butter and ice cream.

² Presented as median with interquartile range because of skewed distribution.

³ Defined as milk and milk products with a fat content < 2.0 g/100 g or cheese products with a fat content < 20 g/100 g.

⁴ Defined as milk and milk products with a fat content > 3.5 g/100 g or cheese products with a fat content > 20 g/100 g.

⁵ Included all fermented dairy products such as buttermilk, yogurts, curds, and cheese products.

⁶ Included all kind of milk, yogurt, coffee creamer, curd, pudding, porridge, custard and whipped cream (both low-fat and high-fat).

⁷ Included soft cheese, hard cheese, and cheese spreads (both low-fat and high-fat).

associated with a 7% lower risk for hypertension (Multivariate HR, 0.93; 95% CI, 0.89, 0.98). When we examined the association with dairy products stratified by low or high fat-content, the significant inverse association was primarily limited to low-fat dairy intake (Table 3.2). Also, intake of milk and milk products was inversely related to hypertension (P-trend: 0.009), whereas intake of fermented dairy products tended to be inversely associated to hypertension (P-trend: 0.08). We observed no association for cheese products (Table 3.2).

Table 3.2 Hazard ratios (95% CI) for 2-y incidence of hypertension by energy-adjusted dairy intake in 2245 Dutch adults aged 55y and over.

	Quartiles of energy-adjusted dairy intake				P-trend ⁷
	1	2	3	4	
<i>n</i>	561	561	562	561	
Total dairy ¹					
Median intake, g/d	164	325	472	691	
Cases	190	168	150	156	
Age and sex adjusted	1.00	0.86 (0.70, 1.06)	0.70 (0.56, 0.86)	0.76 (0.62, 0.94)	0.006
Model 1	1.00	0.82 (0.67, 1.02)	0.66 (0.53, 0.83)	0.74 (0.59, 0.91)	0.003
Model 2	1.00	0.82 (0.67, 1.02)	0.67 (0.54, 0.84)	0.76 (0.61, 0.95)	0.008
Low-fat dairy ²					
Median intake, g/d	21	175	325	561	
Model 2	1.00	0.75 (0.60, 0.92)	0.77 (0.63, 0.96)	0.69 (0.56, 0.86)	0.003
High-fat dairy ³					
Median intake, g/d	28	80	131	272	
Model 2	1.00	0.96 (0.76, 1.21)	1.20 (0.95, 1.51)	1.02 (0.80, 1.29)	0.77
Fermented dairy ⁴					
Median intake, g/d	31	97	188	357	
Model 2	1.00	0.97 (0.79, 1.20)	0.80 (0.64, 1.00)	0.85 (0.68, 1.06)	0.08
Milk & milk products ⁵					
Median intake, g/d	127	288	434	651	
Model 2	1.00	0.92 (0.75, 1.14)	0.66 (0.53, 0.83)	0.79 (0.63, 0.99)	0.009
Cheese & cheese products ⁶					
Median intake, g/d	15	28	39	58	
Model 2	1.00	1.18 (0.95, 1.47)	1.18 (0.95, 1.46)	0.95 (0.75, 1.21)	0.60

¹ Included all dairy products except butter and ice cream.

² Defined as milk and milk products with a fat content <2.0 g/100 g or cheese products with a fat content <20 g/100 g.

³ Defined as milk and milk products with a fat content >3.5 g/100 g or cheese products with a fat content >20 g/100 g.

⁴ Included all fermented dairy products such as buttermilk, yogurts, curds, and cheese products.

⁵ Included all kind of milk, yogurt, coffee creamer, curd, pudding, porridge, custard and whipped cream (both low-fat and high-fat).

⁶ Included soft cheese, hard cheese, and cheese spreads (both low-fat and high-fat).

⁷ Linear trend across quartiles was tested by entering the median values within quartiles into the model as covariate.

Model 1: Adjusted for age (continuous), sex, BMI (continuous), smoking (yes/no), educational level (3 categories), total energy intake (continuous), and alcohol consumption (3 categories)

Model 2: as model 1 with additional adjustment for intake of vegetables, fruit (including fruit juices), meat, bread, coffee, and tea (all continuous)

The association between dairy intake and hypertension did not significantly vary by sex. Hazard ratios for low-fat dairy in the upper vs lower quartiles of intake were 0.61 (0.43, 0.88) for men and 0.74 (0.55, 0.98) for women (P-trend: 0.03 and 0.04, respectively). When we stratified our analyses by BMI (BMI <25 vs \geq 25 kg/m²), hazard ratios across increasing categories of low-fat dairy intake were 1.00, 0.82 (0.58, 1.15), 0.83 (0.58, 1.18), 0.98 (0.69, 1.38) in lean subjects (P-trend: 0.92) and 1.00, 0.69 (0.63, 0.91), 0.71 (0.54, 0.94), 0.55 (0.41, 0.73) in overweight subjects (P-trend: <0.001).

Dairy intake and 6-year incidence of hypertension

After 6 y of follow-up, 984 subjects suffered from hypertension. The associations with hypertension were attenuated with hazard ratios of 0.81 (0.70, 1.01) both for total and low-fat dairy in the upper vs lower quartiles of intake (P-trend: 0.07 and 0.09, respectively), whereas fermented dairy intake was no longer related to hypertension risk (P-trend: 0.92, **Table 3.3**).

When we stratified our analyses by sex, hazard ratios across increasing categories of low-fat dairy intake were 1.00, 0.82 (0.64, 1.07), 0.79 (0.60, 1.05), 0.75 (0.56, 0.99) in men (P-trend: 0.04) and 1.00, 0.90 (0.69, 1.17), 0.82 (0.64, 1.06), 0.90 (0.70, 1.16) in women (P-trend: 0.45). The association between low-fat dairy intake and 6 y incidence of hypertension stratified by BMI did only vary in the highest categories of dairy intake: hazard ratios across increasing categories of low-fat dairy intake were 1.00, 0.80 (0.60, 1.05), 0.72 (0.54, 0.97), 1.01 (0.77, 1.33) in lean subjects (P-trend: 0.99) and 1.00, 0.90 (0.71, 1.15), 0.84 (0.66, 1.08), 0.76 (0.59, 0.97) in overweight subjects (P-trend: 0.03).

Discussion

In this prospective cohort study among 2245 older Dutch adults, we found an inverse association of total dairy and low-fat dairy intake with risk of developing hypertension. Larger risk reductions were observed after 2 y than 6 y of follow-up and among subjects with overweight. Consumption of milk and milk products was inversely associated with incident hypertension, whereas high-fat dairy and cheese were not related to risk of hypertension. Although fermented dairy tended to be inversely associated with incident hypertension after 2 y, this association was no longer present after 6 y.

Several strengths and limitations of our study need to be addressed. In the Netherlands, a variety of dairy products is commonly consumed (median intake around 400 g/d in this study), which enabled us to examine the association of different dairy foods with hypertension over a broad range of intake. A possible limitation is misclassification of dairy intake, which was based on self-report. Assessment of dietary intake in our study was validated against multiple food records. Although the food frequency questionnaire was not specifically validated for dairy intake, high correlations were found for protein

Table 3.3 Hazard ratios (95% CI) for 6-y incidence of hypertension by energy-adjusted dairy intake in 2245 Dutch adults aged 55y and over.

	Quartiles of energy-adjusted dairy intake				P-trend ⁷
	1	2	3	4	
<i>n</i>	561	561	562	561	
Total dairy ¹					
Median intake, g/d	164	325	472	691	
Cases	260	241	239	244	
Age and sex adjusted	1.00	0.85 (0.71, 1.02)	0.80 (0.67, 0.96)	0.85 (0.71, 1.02)	0.08
Model 1	1.00	0.83 (0.69, 0.99)	0.77 (0.64, 0.93)	0.82 (0.69, 0.99)	0.05
Model 2	1.00	0.82 (0.68, 0.98)	0.78 (0.64, 0.93)	0.84 (0.70, 1.01)	0.09
Low-fat dairy ²					
Median intake, g/d	21	175	325	561	
Model 2	1.00	0.86 (0.72, 1.04)	0.81 (0.67, 0.97)	0.84 (0.70, 1.01)	0.07
High-fat dairy ³					
Median intake, g/d	28	80	131	272	
Model 2	1.00	0.90 (0.75, 1.09)	0.94 (0.77, 1.14)	0.93 (0.76, 1.13)	0.66
Fermented dairy ⁴					
Median intake, g/d	31	97	188	357	
Model 2	1.00	0.91 (0.76, 1.09)	0.90 (0.74, 1.08)	0.96 (0.80, 1.16)	0.92
Milk & milk products ⁵					
Median intake, g/d	127	288	434	651	
Model 2	1.00	0.81 (0.67, 0.97)	0.75 (0.63, 0.91)	0.83 (0.69, 1.01)	0.07
Cheese & cheese products ⁶					
Median intake, g/d	15	28	39	58	
Model 2	1.00	0.99 (0.82, 1.19)	0.96 (0.80, 1.16)	0.98 (0.81, 1.18)	0.76

¹ Included all dairy products except butter and ice cream.

² Defined as milk and milk products with a fat content <2.0 g/100 g or cheese products with a fat content <20 g/100 g.

³ Defined as milk and milk products with a fat content >3.5 g/100 g or cheese products with a fat content >20 g/100 g.

⁴ Included all fermented dairy products such as buttermilk, yogurts, curds, and cheese products.

⁵ Included all kind of milk, yogurt, coffee creamer, curd, pudding, porridge, custard and whipped cream (both low-fat and high-fat).

⁶ Included soft cheese, hard cheese, and cheese spreads (both low-fat and high-fat).

⁷ Linear trend across quartiles was tested by entering the median values within quartiles into the model as covariate.

Model 1: Adjusted for age (continuous), sex, BMI (continuous), smoking (yes/no), educational level (3 categories), total energy intake (continuous), and alcohol consumption (3 categories)

Model 2: as model 1 with additional adjustment for intake of vegetables, fruit (including fruit juices), meat, bread, coffee, and tea (all continuous)

and calcium ($r = 0.6-0.7$)¹⁸. Moreover, the Netherlands is a country where dairy products form part of a traditional diet. About 75% of the dairy products is consumed at typical moments during the day, i.e. milk and milk products and cheese during two bread meals (breakfast and often lunch) and dairy desserts after a warm meal (often dinner)²⁰. We, therefore, assume that subjects were able to recall their dairy intake in such way that they could be adequately ranked. Errors in the measurement of dairy intake are likely to bias toward the null hypothesis (no association between dairy intake and hypertension), resulting in an underestimation of the true magnitude of the association. We observed somewhat weaker associations when examining baseline dairy intake in relation to 6-y risk compared to 2-y risk of hypertension which we cannot fully explain. Dairy intake may better predict short-term (2 y) than long-term (6 y) onset of hypertension. This raises the hypothesis that dairy may slow the progression rather than prevent the development of hypertension in certain individuals. Alternatively, participants could have changed their diet between 2 and 6 y of follow-up resulting in attenuation due to misclassification of exposure. Finally, it could be possible that individuals who are (most) susceptible for a beneficial effect of dairy may already benefit in 2 years, while individuals who do not 'respond' within 2 years will also not 'respond' in 6 years. For example, development of hypertension may be secondary to the development of another disease which is not responsive to dietary intake.

The extensive data collection of the Rotterdam Study allows control for many potential confounders. We had no data on physical activity which is a known determinant of blood pressure. Although we did adjust for total energy and body mass index as surrogate markers for physical activity, we cannot exclude residual confounding. A questionnaire on physical activity has been added to the Rotterdam Study in 1997 (second follow-up round) and data has recently become available for a subpopulation ($n \sim 600$ for the present study). Dairy intake was not clearly related to physical activity expressed as MET-h/w in this subpopulation; 102 ± 52 h/w ($n = 207$), 109 ± 55 h/w ($n = 208$), 108 ± 50 h/w ($n = 221$), and 103 ± 48 h/w (208) for the consecutive categories of total dairy intake (P-trend: 0.40).

Several observational studies have shown an inverse association between dairy intake and blood pressure or hypertension⁹⁻¹⁶. These studies have been conducted in children¹⁶, young adults^{13, 15}, middle-aged^{10, 12, 14} or older adults¹⁴. Our study in a general older population largely agrees with previous reports, including the study by Wang et al. who found that total and low-fat dairy were associated with reduced risk of hypertension of about 10-15% in middle-aged and older women¹⁴. We observed that risk reductions were more pronounced among individuals with overweight compared to lean individuals. This is in line with the study by Pereira et al. who observed significant associations only among overweight individuals¹³. In contrast with previous studies, we observed no clear

'dose-response relation' between dairy intake and hypertension, but rather a 'threshold effect'. While especially a low dairy intake was associated with an increased risk of hypertension, risk estimates across higher dairy intakes were more or less similar.

Dairy intake is a rather heterogeneous exposure and little is known about specific types of dairy foods in relation to blood pressure. Although both total and low-fat dairy were inversely related to hypertension risk, the effect of total dairy is probably due to its high correlation with low-fat dairy ($r = 0.77, p < 0.0001$). Most of the dairy products in the DASH diet were in the form of low-fat milk (74%) and fat-free yogurt (18%)²³. Observational studies that examined the association with dairy products stratified by fat content mainly observed beneficial effects of low-fat dairy rather than high-fat dairy^{12,14}, although results are not conclusive^{11,16}. It is not clear why especially low-fat dairy products could affect blood pressure, but it is speculated that saturated fats may mitigate the beneficial effects of other dairy components. This would also explain the weaker or null association with high-fat dairy food groups (i.e. high-fat dairy, fermented dairy and cheese) in our study. Another concern with respect to dairy intake is related to (high) sodium levels in all 'salted' cheese products which is a well-established risk factor for hypertension^{24,25}. Because our cheese category consisted mainly of regular high-fat (hard/salted) cheese products, this probably explains why we did not observe an association for this category. Other studies that examined the relation between cheese and blood pressure observed no association either inverse or direct^{11,13,15}, except for Ruidavets et al. who showed that fresh cheese was inversely related to blood pressure¹⁰.

The mechanism via which dairy may reduce blood pressure remains to be established. Dairy is rich in protein and bioactive peptides which might exert a beneficial effect on blood pressure, e.g. by inhibiting the angiotensin-1-converting enzyme, modulating endothelium function or by affecting body weight. Likewise, minerals in dairy foods, such as calcium, magnesium and potassium, are important for blood pressure regulation and have been associated with a reduced risk of hypertension⁴⁻⁸. A meta-analysis of 40 randomized controlled trials showed that calcium supplementation may significantly reduce blood pressure, especially in populations with a habitually low calcium intake²⁶. Moreover, studies addressing this topic have shown greater consistency using whole foods than using non-dietary calcium supplements⁶, suggesting the importance of the total dietary pattern.

Blood pressure is a major risk factor for cardiovascular diseases. A population-wide shift in systolic blood pressure distribution of 2-5 mm Hg has been translated into important reductions in mortality due to stroke and coronary heart disease and in total mortality^{24,27}. The results from our study suggest that low-fat dairy could play a role in the prevention of hypertension in a general older population.

Conflict of interest

None.

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Chapter 4

Lactotripeptides show no effect on human blood pressure:
Results from a double-blind randomized controlled trial

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Abstract

Milk derived peptides with ACE-inhibiting properties may have antihypertensive effects in humans. We conducted a randomized double-blind placebo-controlled trial to examine the blood pressure lowering potential of 2 ACE-inhibiting lactotriptides, i.e. Isoleucine-Proline-Proline and Valine-Proline-Proline. We included 135 Dutch subjects with elevated systolic blood pressure who were otherwise healthy and who received no current antihypertensive treatment. After a 2-week run-in period on placebo, subjects randomly received a daily dose of 200 mL dairy drink with 14 mg lactotriptides obtained by concentrating fermented milk, enzymatic hydrolysis, or chemical synthesis, or placebo for 8 weeks, followed by a 2-week wash-out. The primary outcome was 8-week change in office systolic blood pressure. Secondary outcomes were change in diastolic blood pressure, home blood pressure, 24-hour ambulatory blood pressure, plasma ACE-activity, and plasma angiotensin II. Blood pressure at baseline was on average 142/84 mm Hg. Lactotriptides did not significantly change systolic blood pressure ($P = 0.46$) or diastolic blood pressure ($P = 0.31$) compared with placebo. The mean difference (95% CI) in systolic blood pressure response between treatment and placebo was 2.8 mm Hg (-2.6, 8.2) for concentrated fermented milk lactotriptides, -0.5 mm Hg (-6.0, 5.0) for enzymatic lactotriptides and 1.6 mm Hg (-3.9, 6.9) for synthetic lactotriptides. Treatment neither had a significant effect on secondary outcome measures. In conclusion, the present study does not support the hypothesis of a blood pressure lowering effect of the lactotriptides Isoleucine-Proline-Proline and Valine-Proline-Proline.

Introduction

There is growing evidence for a beneficial effect of dairy intake on blood pressure¹. In the Dietary Approaches to Stop Hypertension (DASH) trial larger blood pressure reductions were achieved when low-fat dairy was added to a healthy fruit and vegetable diet². Dairy food is rich in protein and calcium, which could have beneficial effects on blood pressure³⁻⁵. In addition, fermented milks and casein hydrolysates decreased blood pressure in a number of Japanese and Finnish trials in (mildly) hypertensive human subjects⁶⁻¹⁴. Studies of fermented milk showed reductions of 1.5 to 11.0 mm Hg for systolic blood pressure (SBP) and 0.5 to 6.8 mm Hg for diastolic blood pressure (DBP), compared with placebo^{6-8, 10, 12-14}. The proposed underlying antihypertensive mechanism of these bioactive peptides is, at least partly, ACE-inhibition. Isoleucine-Proline-Proline (IPP) and Valine-Proline-Proline (VPP), 2 lactotripeptides (LTP), are considered the most promising ACE-inhibiting bioactive peptides. IPP and VPP can be derived from milk casein by the action of the proteolytic system of lactic acid bacteria (*Lactobacillus helveticus*). Recently, an enzymatic method using an *Aspergillus oryzae* protease has been developed to generate IPP and VPP from milk casein¹⁵. This casein hydrolysate decreased blood pressure in Japanese hypertensive subjects^{9, 11}. Both fermented and enzymatically hydrolyzed milk contain a large variety of peptides. Therefore, supplementation with chemically synthesized IPP and VPP may be more appropriate for studying specific effects of these peptides. An antihypertensive effect of chemically synthesized IPP and VPP has been demonstrated in spontaneously hypertensive rats^{16, 17}, but not yet in humans.

We performed a randomized double-blind placebo-controlled trial to examine whether daily intake of 14 mg LTP, obtained by concentrating fermented milk, enzymatic hydrolysis, or chemical synthesis, would influence blood pressure over an 8-week period in 135 Dutch subjects with elevated blood pressure who received no current antihypertensive treatment.

Subjects and Methods

Subjects

Men and women (35 to 70 y) with elevated SBP were recruited from the Dutch population in collaboration with general practitioners. During a screening phase 2 consecutive blood pressure measurements were performed at the study center with at least 48 hours in between. Subjects with elevated blood pressure, defined as SBP ≥ 140 mm Hg (mean of last 3 of 4 measurements, taken after 15-minute rest at 2-minute intervals) on both occasions, were invited for further screening. Subjects were excluded if they reported metabolic diseases, gastrointestinal disorders, cardiovascular disease, or renal disease. Plasma glucose, liver function (ALAT, ASAT, γ -GT), renal function (ureum and creatinine), and hematology (WBC, RBC, hemoglobin, hematocrit, platelet count) had to be normal based on the judgement of general practitioners, who evaluated these data for their own

patients. Additional exclusion criteria were: antihypertensive medication less than 3 weeks before screening; body mass index $>32 \text{ kg/m}^2$; weight loss ($>10\%$) during 6 months before screening; cow milk allergy or lactose intolerance; pregnancy or lactation; blood donation; alcohol abuse; and participation in night shift work. The Medical Ethics Committee of Wageningen University approved the study and all subjects gave written informed consent. In addition, agreement was obtained from each subject's general practitioner. The study was conducted in accordance with the principles of the Declaration of Helsinki.

Design and randomization procedure

We performed a randomized double-blind placebo-controlled trial with 4 parallel arms. Baseline measurements took place at the end of a 2-week run-in period during which subjects consumed the placebo product (i.e., dairy drink without LTP) and followed study guidelines as described below. Two independent persons, not involved in the conduct of the study, randomly allocated eligible subjects to 1 of the 3 treatment groups or placebo by means of computer-generated randomization codes stratified by prestudy use of antihypertensive medication (yes versus no), baseline SBP ($<150 \text{ mm Hg}$, $150 \text{ to } 160 \text{ mm Hg}$, or $>160 \text{ mm Hg}$), and gender. Subjects took one study drink each morning for an 8-week period. An additional blood pressure measurement was performed after a 2-week wash-out period.

Dairy drinks

Test products were low-fat yogurt drinks that were ready-to-drink and provided by Unilever Food & Health Research Institute, Vlaardingen, the Netherlands. **Table 4.1** shows the nutritional composition of the products. The 3 treatment products contained IPP and VPP, which are lactotripeptides (LTP) generated by concentrating fermented milk, enzymatic hydrolysis, or chemical synthesis. Daily supply of dairy drink per subject was 200 mL, containing 4.2 to 5.4 mg IPP and 5.0 to 5.8 mg VPP, which equals 14 mg LTP equivalents in all active arms. ACE-inhibitory activity of the test products was analyzed according to Matsui et al¹⁸. The fermented and enzymatic LTP drinks had an ACE-inhibition of 50% using a concentration of 0.025% protein of the added active powder. The synthetic LTP drink had an ACE-inhibition of 45% using a 1000 times lower protein concentration. The placebo drink did not show any ACE-inhibition. Additionally, all test products contained orange juice concentrate, sugars, flavors for taste, pectin for stability, and water. Test products were similar in appearance, color, taste and smell, and were provided in nontransparent white cups every 2 weeks. Two research assistants, not otherwise involved in the study, labeled and supplied the test products. Compliance was assessed by counting all empty and full cups that were returned by subjects. Research staff and subjects remained blinded towards the type of treatment during the study and data analysis.

Table 4.1 Nutrient composition per 100 g of dairy drinks (daily dose¹)

Nutrient	Fermented LTP ²	Enzymatic LTP	Synthetic LTP	Placebo
Calorie, kcal	64 (128)	61 (122)	60 (120)	61 (122)
Protein, g	2.6 (5.2)	2.8 (5.6)	2.7 (5.4)	2.7 (5.4)
Carbohydrates, g	13.4 (26.8)	12.5 (25.0)	12.4 (26.8)	12.5 (25.0)
Fat, g	-	-	-	-
Calcium, mg	95 (190)	71 (142)	71 (142)	75 (150)
Sodium, mg	34 (68)	45 (90)	32 (64)	35 (70)
Potassium, mg	146 (292)	147 (294)	147 (294)	153 (306)
Magnesium, mg	13 (26)	9 (18)	9 (18)	9 (18)
Lactotripeptides				
IPP ³ , mg	2.1 (4.2)	2.7 (5.4)	2.6 (5.2)	-
VPP ⁴ , mg	2.9 (5.8)	2.5 (5.0)	2.5 (5.0)	-

¹ Subjects took 200 mL daily.

² LTP indicates lactotripeptides (IPP and VPP).

³ IPP, Isoleucine-Proline-Proline.

⁴ VPP, Valine-Proline-Proline.

Study procedures

Height was measured to the nearest 0.5 cm using a wall-mounted stadiometer. Body weight was measured without shoes and heavy clothing at each visit (Seck Bascule MT), and body mass index was computed (kg/m²). At baseline, subjects filled out a 21-item food frequency questionnaire for intake of dairy products from which daily protein and calcium intake were computed using Dutch food composition tables (NEVO, The Hague, 2001).

Subjects recorded consumption of test products, deviations from the study protocol, episodes of illness or hospitalization, use of medication, and health complaints in a diary. Adverse events were coded by the study physician (EGS) according to the International Classification of Diseases (ICD10).

Subjects were asked to maintain their habitual dietary and lifestyle pattern during the study. In particular they were asked not to change intake of salt and salty foods, alcoholic beverages, and fermented products. Subjects were instructed to consume the test product daily at breakfast. At the day before a test day at the study center, subjects were asked to fast from 8 PM onwards. They were allowed to consume breakfast and test product on test days, but at a fixed point in time at least 2 hours before their appointment at the study center.

Blood pressure measurements

Blood pressure measurements were performed at the study center (office BP) and by subjects themselves at home (home BP). In 58 subjects (43%) we additionally obtained

24-hour ambulatory blood pressure readings (ABPM). Subjects remained blinded toward all blood pressure values until data analysis was completed. The primary outcome measure was 8-week change in SBP measured at the study center.

Trained staff measured blood pressures at the study center during screening, at baseline, after 4 weeks, after 8 weeks, and after the 2-week wash-out period. After a 15-minute rest, 4 measurements with 2-minute intervals were performed on the dominant arm in sitting position, using an automated blood pressure device with an appropriately sized cuff (Omron HEM-907). The first reading was discarded, and 3 subsequent readings were averaged. Blood pressure measurements were repeated after 1 day and values of both occasions were averaged. All blood pressure measurements were performed between 8 and 12 AM at a fixed time for each individual.

Subjects performed measurements at home on the same days as the study center visits according to the same standardized procedures. They measured blood pressure using a blinded automated device (Stabil-O-Graph) at 3 fixed time points: in the morning between awakening and breakfast, before lunch, and before bedtime. Readings were stored and downloaded afterwards on a computer at the study center. A group of 58 subjects volunteered for 24-hour ABPM at baseline, week 4 and week 8. Measurements were performed from 12 AM till 12 AM next day with 30-minutes intervals during the day (7 AM to 11 PM) and 60-minutes intervals during the night (11 PM to 7 AM), using a blinded automated device (Space Lab type 90217). Readings were stored and downloaded afterward on a computer at the study center. Mean 24-hour, day (9 AM to 9 PM), and night (1 AM to 6 AM) values were calculated.

Laboratory determinations

Research nurses performed blood sampling at screening, baseline and after 4 and 8 weeks of intervention. During screening, they used venipuncture. During the study, the nurses inserted a catheter into the antecubital vein, after which the subject rested for 20 minutes before blood was taken by a syringe. The collected blood was directly processed on ice and stored at -80°C . For determination of ACE-activity blood was collected in Li-heparin tubes. Plasma ACE-activity was assessed in duplicate by use of a fluorescence assay (BiPharma, Fujirebio Diagnostics Inc), with an intraassay coefficient of variation of 3.8% and an interassay coefficient of variation of 5.8%. For the determination of Angiotensin II blood was collected in chilled EDTA tubes containing ACE and renin inhibitors to prevent conversion. Before determination, plasma was treated with Sep-Pak C18 columns. Subsequently, plasma concentration of Angiotensin II was assessed in duplicate by Radioimmunoassay (Penninsula Laboratories Inc Europe), with an intraassay coefficient of 4.6% and interassay coefficient of 7.7%. All samples of each subject were analyzed in one run. Plasma glucose, liver enzymes, indicators of

renal function and hematology were assessed during screening and after 8 weeks using standard laboratory methods.

At baseline and after 8 weeks, 24-hour urine collections were obtained for determination of sodium and potassium excretion. Creatinine was measured to check completeness of urine collection. Urinary volume and collection times were used to calculate 24-hour excretion of sodium and potassium.

Statistical analysis

Double-data entry was performed and discrepancies were solved. Treatment codes were broken after blind data analysis. Data were analyzed according to the intention-to-treat principle.

Values reported in text and tables are means with standard errors (SE) or 95%-confidence intervals (95% CI), unless stated otherwise. Response to treatment was defined as change in blood pressure from baseline. Blood pressure data were analyzed using analysis of variance (ANOVA). Subsequently, the Dunnett test was used to compare mean changes within different treatment groups with that of the control group. Data analysis was repeated after exclusion of noncompliant subjects who consumed less than 80% of the test products.

To examine whether dairy calcium or protein in the background diet would modify blood pressure response to intake of LTP (all 3 treatment groups combined), we performed a post-hoc analysis in 118 subjects who completed the food frequency questionnaire. For this purpose, blood pressure effects were reexamined after stratification for dairy calcium intake (below versus above the median of 726 mg per day) and for dairy protein intake (below versus above the median of 24 g per day).

Analyses were performed using SPSS 11.0.1 for Windows. Two-sided probability values below 0.05 were considered statistically significant. The present study had a power of 83% for detecting a 5 mm Hg difference in systolic blood pressure response, with $\alpha=0.05$ (2-sided).

Results

Subjects and compliance

We enrolled 135 subjects in the trial. One subject dropped out after 3 weeks for personal reasons not related to the study. **Figure 4.1** shows the number of subjects screened, excluded, and randomized. There were no relevant differences in subject's characteristics, including blood pressure, among the groups (**Table 4.2**). Compliance was satisfactory, with only 4 subjects consuming less than 80% of the test products.

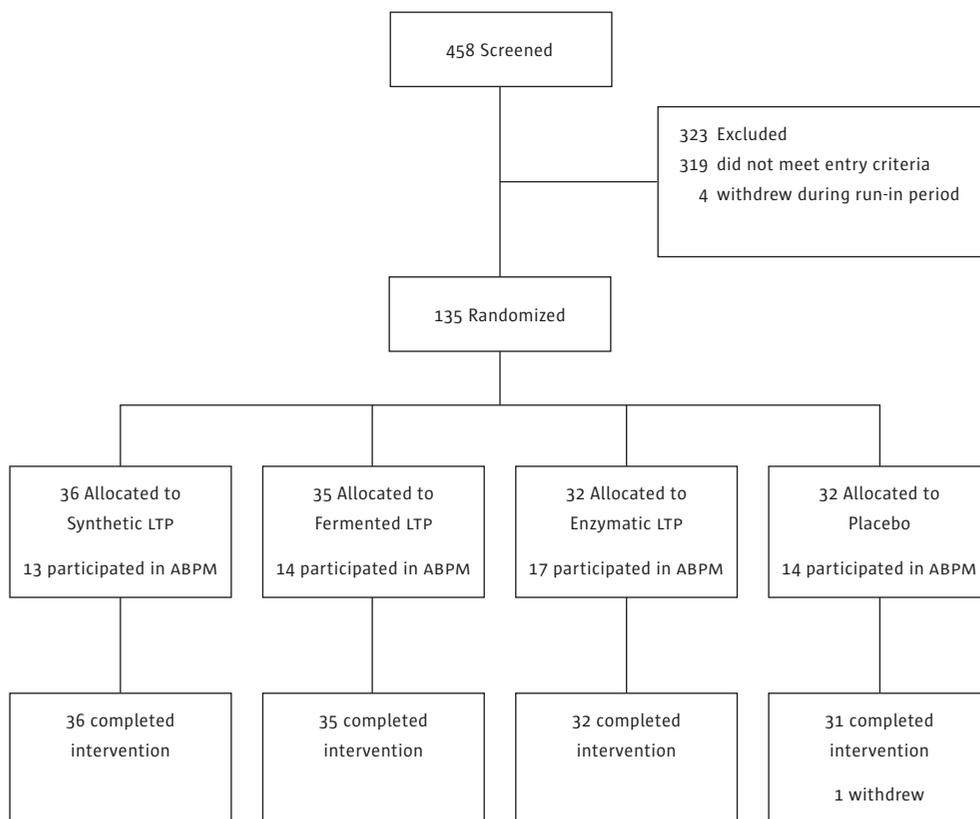


Figure 4.1 Flow chart of a double-blind, randomized, placebo-controlled trial of lactotripeptides and blood pressure in 135 Dutch subject with untreated elevated blood pressure.

LTP, lactotripeptides (IPP and VPP)

ABPM, 24h ambulatory blood pressure measurement

Blood pressure

Figure 4.2 shows mean office SBP and DBP in the 4 groups throughout the study. There was no significant effect of LTP treatment on SBP ($P = 0.46$). The mean difference (95% CI) in 8-week change between treatment and placebo was 2.8 mm Hg (-2.6, 8.2) for concentrated fermented milk based LTP, -0.5 mm Hg (-6.0, 5.0) for enzymatic LTP and 1.6 mm Hg (-3.9, 6.9) for synthetic LTP (**Table 4.3**). LTP treatment did neither affect DBP ($P = 0.31$, **Table 4.3**). Exclusion of 4 noncompliant subjects yielded similar results (data not shown).

Mean home blood pressure at baseline in the group as a whole was 153/95 mm Hg, i.e., 149/94 mm Hg in the morning, 150/94 mm Hg at noon and 158/98 mm Hg in the evening. Values were similar for treatment and placebo groups. LTP treatment did not significantly affect home blood pressure (all probability values >0.3). The mean difference (95% CI) in home SBP response between treatment and placebo was -0.9 mm

Table 4.2 Baseline characteristics of 135 subjects with untreated elevated blood pressure, by treatment

Characteristic	Fermented LTP ¹ (n = 35)	Enzymatic LTP (n = 32)	Synthetic LTP (n = 36)	Placebo (n = 32)
Age, y	58.8 ± 9.1 ²	54.2 ± 8.8	59.5 ± 8.2	58.9 ± 10.3
Sex, male/female	23/12	22/10	23/13	20/12
Blood pressure category, n (%) ³				
Optimal	1 (3)	1 (3)	2 (6)	1 (3)
Normal	4 (11)	6 (19)	4 (11)	5 (26)
High normal	11 (31)	7 (22)	10 (28)	9 (28)
Hypertension				
Stage 1	17 (49)	14 (44)	16 (44)	14 (44)
Stage 2-4	2 (6)	4 (13)	4 (11)	3 (10)
Resting heart rate, beats/min	68.6 ± 8.6	74.9 ± 7.5	68.1 ± 8.0	67.5 ± 7.5
Pre-study use of antihypertensive medication (past 3 mo), n (%) ⁴	5 (14.3)	2 (6.3)	3 (8.3)	1 (3.1)
Body weight, kg	81.8 ± 12.3	84.7 ± 11.1	83.2 ± 12.2	83.5 ± 13.5
Body Mass Index, kg/m ²	26.9 ± 2.6	26.8 ± 2.8	27.0 ± 2.9	26.8 ± 2.9
Current cigarette smoking, n (%) ⁴	3 (9)	3 (9)	5 (14)	3 (9)
Glucose, mmol/L ⁴	6.0 ± 0.1	5.8 ± 0.1	6.0 ± 0.1	5.9 ± 0.1
Dairy protein intake, g/d ⁴	24 ± 10	25 ± 10	26 ± 13	29 ± 17
Dairy calcium intake, mg/d ⁴	738 ± 316	807 ± 334	811 ± 419	926 ± 589

¹ LTP indicates lactotripeptides (IPP and VPP).

² Values are mean (±SD) or percentages.

³ None of the subjects used antihypertensive medication at baseline. Blood pressure categories defined as follows: Optimal, SBP <120 mm Hg and DBP <80 mm Hg; Normal, 120-129 mm Hg for SBP or 80-84 mm Hg for DBP; High normal, 130-139 mm Hg for SBP or 85-89 mm Hg for DBP; Hypertension – stage 1 (mild), 140-159 mm Hg for SBP or 90-99 mm Hg for DBP; Hypertension – stage 2-4, ≥160 mm Hg for SBP or ≥110 mm Hg for DBP.

⁴ Assessed during screening.

Hg (−6.1, 4.4) for concentrated fermented milk based LTP, −1.8 mm Hg (−7.2, 3.6) for enzymatic LTP and 0.6 mm Hg (−4.7, 5.9) for synthetic LTP. Detailed data on home blood pressure responses throughout the day are tabulated in the online supplement to this paper (**Supplemental table 4.1**). Mean ABPM in 58 subjects was 144/87 mm Hg at baseline, with higher values during the day (150/90 mm Hg) than the night (123/72 mm Hg). Changes in 24-hour SBP during intervention in the 3 active arms were not significantly different from placebo (all $P > 0.3$): the mean difference (95% CI) in 24-hour SBP response (12 AM to 12 AM) was 4.6 mm Hg (−3.1, 12.3) for concentrated fermented milk based LTP, 2.6 mm Hg (−4.7, 9.9) for enzymatic LTP and 4.2 mm Hg (−3.6, 12.1) for synthetic LTP. Results were similar for daytime and nighttime SBP, and for different measures of DBP. Additional ambulatory 24-hour SBP curves for each group are shown in the online supplement to this paper (**Supplemental figures 4.1 and 4.2**). Restricting the analysis to 46 compliant subjects (i.e., subjects with over 70% readings and more than 20 hours on each occasion) yielded similar results.

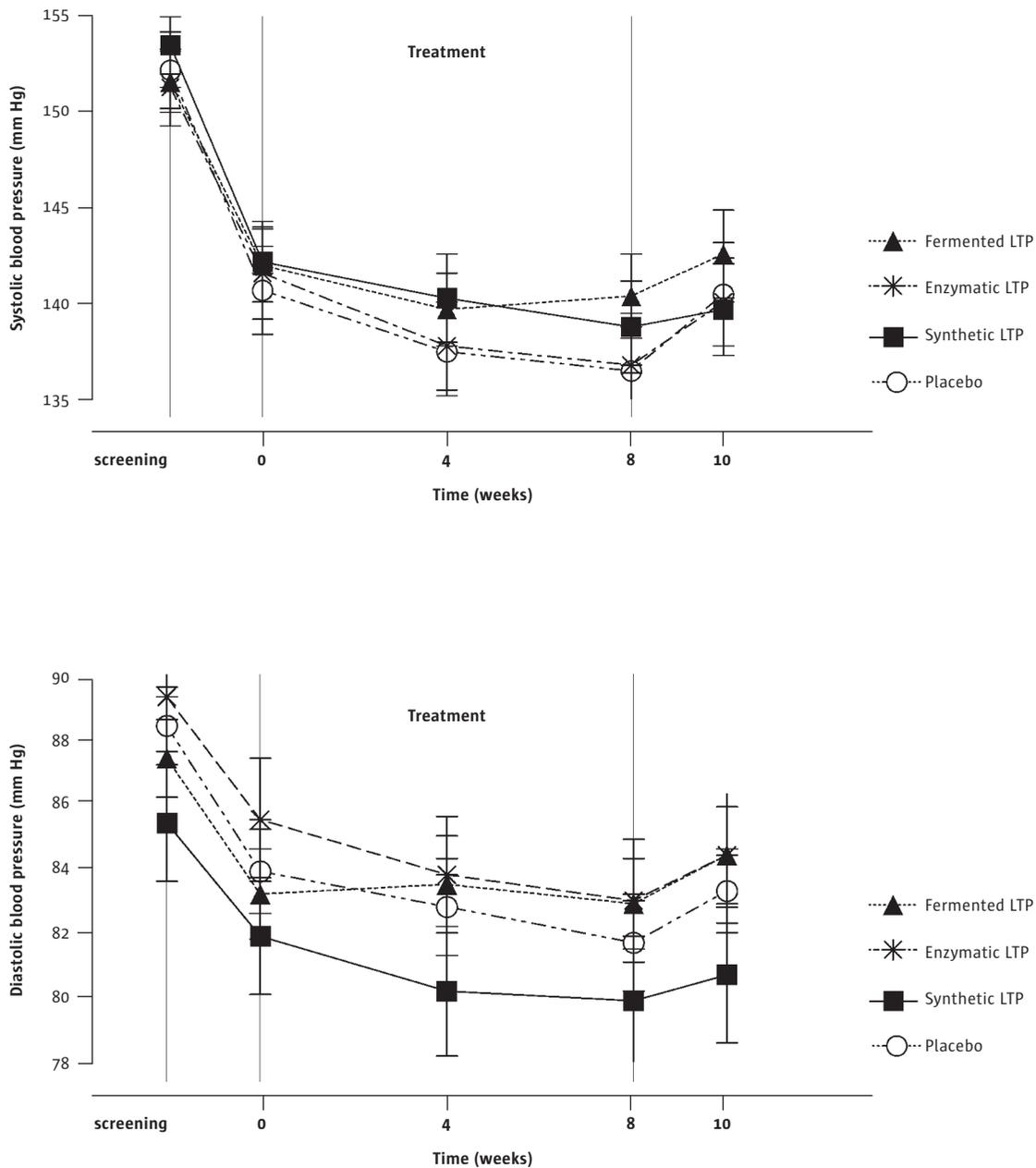


Figure 4.2 Mean values (SE) for office systolic and diastolic blood pressure during run-in, intervention and wash-out in 135 randomized subjects in the three treatment groups and the placebo group.
 SBP, systolic blood pressure
 DBP, diastolic blood pressure
 LTP, lactotripeptides (IPP and VPP)

Table 4.3 Office blood pressure during intervention in 135 subjects with untreated elevated blood pressure, by treatment

Outcome	Fermented LTP ¹ (n = 35)	Enzymatic LTP (n = 32)	Synthetic LTP (n = 36)	Placebo (n = 32)
Systolic BP, mm Hg				
Baseline	142.0 ± 1.9 ²	141.6 ± 2.4	142.2 ± 2.1	140.7 ± 2.3
Week 8 ⁴	140.4 ± 2.2	136.8 ± 2.7	138.8 ± 2.4	136.5 ± 2.3
Change ⁴	-1.6 ± 1.5	-4.8 ± 1.8	-2.8 ± 1.7	-4.3 ± 1.3
Change attributed to treatment, mean difference (95% CI), P-value	2.8 (-2.6, 8.2) P = 0.47 ³	-0.5 (-6.0, 5.0) P = 0.99	1.6 (-3.9, 6.9) P = 0.83	
Diastolic BP, mm Hg				
Baseline	83.2 ± 1.4	85.5 ± 1.9	81.9 ± 1.8	83.9 ± 1.3
Week 8 ⁴	82.9 ± 1.4	83.0 ± 1.9	79.9 ± 2.0	81.7 ± 1.5
Change ⁴	-0.3 ± 1.0	-2.4 ± 0.9	-2.4 ± 1.1	-2.4 ± 0.9
Change attributed to treatment, mean difference (95% CI), P-value	2.2 (-1.2, 5.5) P = 0.30	0.01 (-3.4, 3.5) P = 1.00	-0.01 (-3.4, 3.4) P = 1.00	

¹ LTP indicates lactotripeptides (IPP and VPP).

² Values are mean (±SE).

³ 2-sided P values for the difference from placebo (Dunnett test).

⁴ Missing for 2 subjects (1 synthetic LTP, 1 placebo).

Other outcomes

Body weight did not change significantly during the study. Mean changes (±SD) were: 0.4 ± 0.2 kg for concentrated fermented milk based LTP, 0.0 ± 0.2 kg for enzymatic LTP, 0.1 ± 0.2 kg for synthetic LTP, and 0.0 ± 0.2 kg for placebo. In the group as a whole, baseline urinary 24-hour excretion of sodium, potassium, and creatinine (±SD) were 168.2 ± 5.9 mmol (corresponding to 9.7 g NaCl per day), 91.1 ± 2.6 mmol (3.5 g), and 13.9 ± 4.5 mmol, respectively. The 8-week change in sodium was -17.7 ± 15.0 mmol/L for concentrated fermented milk based LTP, -9.2 ± 10.8 mmol/L for enzymatic LTP, -2.3 ± 14.3 mmol/L for synthetic LTP and +3.9 ± 16.0 mmol/L for placebo. For potassium 8-week changes were -8.8 ± 5.4, -0.2 ± 5.8, -14.0 ± 4.7, and -3.8 ± 7.0 mmol/L, respectively. Changes in sodium and potassium did not significantly differ between treatment and placebo (all P > 0.1).

Mean baseline ACE-activity (±SE) was 30.0 ± 0.7 U/L and remained constant during the study (**Table 4.4**). Baseline plasma angiotensin II concentration was 10.4 ± 0.5 pmol/L and decreased slightly in the concentrated fermented milk based LTP group and in the enzymatic LTP group during intervention, but this was not significantly different from placebo (**Table 4.4**).

Table 4.4 Plasma ACE-activity and angiotensin II concentration in 135 subjects with untreated elevated blood pressure, by treatment

Outcome	Fermented LTP ¹ (n = 35)	Enzymatic LTP (n = 32)	Synthetic LTP (n = 36)	Placebo (n = 32)
ACE-activity, U/L				
Baseline ¹	30.5 ± 1.5	29.1 ± 1.2	30.9 ± 1.4	29.2 ± 1.1
Week 8 ²	30.6 ± 1.5	29.4 ± 1.1	30.7 ± 1.4	28.7 ± 1.1
Change ²	0.1 ± 0.5	0.3 ± 0.4	-0.1 ± 0.4	-0.3 ± 0.5
Change attributed to treatment, mean difference (95% CI), P-value	0.4 (-1.2, 2.0) P = 0.86	0.6 (-1.0, 2.2) P = 0.65	0.2 (-1.4, 1.8) P = 0.98	
Angiotensin II, pmol/L				
Baseline ³	12.2 ± 1.0	10.2 ± 1.0	9.5 ± 0.7	9.8 ± 1.0
Week 8 ⁴	9.6 ± 0.8	8.9 ± 0.6	9.1 ± 0.5	8.8 ± 0.6
Change ⁵	-3.4 ± 0.9	-1.6 ± 0.8	-0.3 ± 0.7	-0.5 ± 1.0
Change attributed to treatment, mean difference (95% CI), P-value	-2.9 (-5.8, 0.1) P = 0.06	-1.1 (-4.2, 2.0) P = 0.73	0.2 (-2.7, 3.1) P = 1.00	

LTP indicates lactotripeptides (IPP and VPP); Values are mean (±SE); 2-sided P values for the difference from placebo (Dunnett test).

¹ Missing for 2 subjects (1 synthetic LTP, 1 placebo).

² Missing for 4 subjects (1 synthetic LTP, 3 placebo).

³ Missing for 3 subjects (1 enzymatic LTP, 1 synthetic LTP, 1 placebo); values below detection limit for 10 subjects (2 fermented LTP, 3 enzymatic LTP, 3 synthetic LTP, 2 placebo).

⁴ Missing for 5 subjects (2 synthetic LTP, 3 placebo); values below detection limit for 11 subjects (5 fermented LTP, 3 enzymatic LTP, 2 synthetic LTP, 1 placebo).

⁵ Missing for 6 subjects (1 enzymatic LTP, 2 synthetic LTP, 3 placebo); values below detection limit for 16 subjects (5 fermented LTP, 5 enzymatic LTP, 3 synthetic LTP, 3 placebo).

Plasma glucose, liver enzymes, indicators of renal function, and hematology were similar in the 4 groups and did not change significantly during the study (data not shown). During the 8 weeks of treatment a few minor adverse events were recorded, but there were no differences according to treatment.

Post-hoc analysis

In 59 subjects with a dairy calcium intake <726 mg/d the change in blood pressure was -3.2/-1.6 mm Hg for all LTP treatments combined versus -5.8/-2.7 mm Hg for the placebo group. In 59 subjects with a higher calcium intake, values were -3.3/-1.7 mm Hg and -3.6/-1.8 mm Hg, respectively. In subjects with a low dairy protein intake (<24 g/d), corresponding values were -3.2/-1.3 mm Hg for LTP versus -6.8/-3.4 mm Hg for placebo, and in subjects with a higher dairy protein intake -3.3/-2.0 mm Hg and -2.6/-1.0 mm Hg, respectively. Probability values for all comparisons were >0.2.

Discussion

This trial showed no antihypertensive effect of the dairy peptides IPP and VPP that were generated by concentrating fermented milk, enzymatic hydrolysis, or chemical synthesis in 135 subjects with high normal and elevated blood pressure. Although SBP and DBP decreased during the study, this was not attributable to effects of IPP and VPP. The results were consistent for office, home and ambulatory blood pressure.

Strengths of this study are its randomized double-blind placebo-controlled design. In addition, compliance was excellent and only 1 subject dropped out. Data were analyzed according to the intention-to-treat principle. Restricting the analysis to compliant subjects yielded similar results. Baseline characteristics, including initial levels of blood pressure, were similar among the 4 groups. Subjects were also able to maintain their habitual level of exercise and dietary pattern during the study, as confirmed by their unchanged body weight and urinary excretion of sodium and potassium.

From most trials in humans it was concluded that IPP and VPP are effective in lowering blood pressure⁶⁻¹⁴. In the majority of studies milk fermented with *Lactobacillus helveticus* was examined in subjects with high normal blood pressure or mild hypertension. The effect on blood pressure, either office, home, or ABPM, ranged from -1.5 to -11.0 mm Hg for SBP and from -0.5 to -6.8 mm Hg for DBP compared with placebo^{6-8, 10, 12-14}. Also, IPP and VPP obtained by enzymatic hydrolysis of casein, using *Aspergillus oryzae* protease, have been shown to lower blood pressure in mild hypertensives^{9, 11}. We considered several possible explanations for the absence of an antihypertensive effect of IPP and VPP in our study. Previous studies that did show an effect had a duration of 4 to 21 weeks and daily doses of IPP and VPP between 2.5 and 52.5 mg. Hence, we conclude that our intervention of 8 weeks with a dose of 14 mg LTP equivalents, containing 4.2 to 5.4 mg IPP and 5.0 to 5.8 mg VPP, should have been sufficient for detecting any effect on blood pressure, if present. Also, the contrast in ACE-inhibitory capacity between the treatment drinks and the placebo should have been sufficient in our study. Secondly, LTP may exert their effect only in subjects with clinically established hypertension. Ninety percent of our subjects had a blood pressure in the high-normal or mildly hypertensive range, after repeated measurements. Some studies that were positive for LTP had higher blood pressure levels at baseline^{7, 12, 14}. It should also be noted that in most studies, despite randomization, initial blood pressure was higher in the intervention than in the control group^{7, 8, 10, 12, 14}. As a result, blood pressure effects attributed to IPP and VPP may have been overestimated. Thirdly, we considered the role of background diet. Our Dutch study population already had a high habitual intake of dairy foods (520 g per day) and calcium from dairy (820 mg per day). Previous studies were almost exclusively performed in Japanese and Finnish subjects, with different dietary patterns. In a post-hoc analysis we examined whether a low or high habitual

intake of dairy protein and calcium could modify blood pressure response to LTP, but this was not the case. Finally, because our subjects were asked to maintain their regular lifestyle or dietary pattern during the study, we cannot exclude a possible beneficial effect of LTP in combination with favorable lifestyle or dietary changes, although we consider this unlikely.

The putative antihypertensive effect of dairy peptides has been related to ACE-inhibition^{17, 19-21}, but we found no consistent changes in plasma ACE-activity or angiotensin II. The potency of ACE-inhibition of most food-derived peptides *in vitro* ranges from 2 to 1000 $\mu\text{mol/L}$, which is over 1000 times weaker than antihypertensive drugs²². Such effects *in vivo* may not have been detectable in our study due to large inter- and intraindividual variation. We did not examine other mechanisms of antihypertensive action, such as a direct relaxation of vascular muscle cells or opioid or antioxidant activities²³.

In conclusion, the present randomized double-blind controlled trial provides no evidence for a blood pressure lowering effect of the lactotripeptides IPP and VPP in human subjects with untreated elevated blood pressure.

Perspectives

There is growing interest in the role of low-fat dairy for the prevention of hypertension. A new line of research in this field focuses on bioactive peptides derived from milk proteins (e.g., IPP and VPP) that could inhibit the angiotensin-converting enzyme (ACE). Previous studies identified IPP and VPP as promising food ingredients for blood pressure control in mildly hypertensive subjects⁶⁻¹⁴. The present double-blind, randomized controlled trial in 135 Dutch subjects with elevated blood pressure, however, provides no evidence for an antihypertensive effect of IPP and VPP. More well-conducted trials in different populations are needed to see whether supplementation with dairy peptides could have a place in the treatment of mild hypertension.

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Conflict of interest

LAJM is working at the Unilever Food & Health Research Institute, Vlaardingen, The Netherlands.

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Supplemental table 4.1 Response in home blood pressure after 8 weeks of intervention in 128 subjects with untreated elevated blood pressure, by treatment

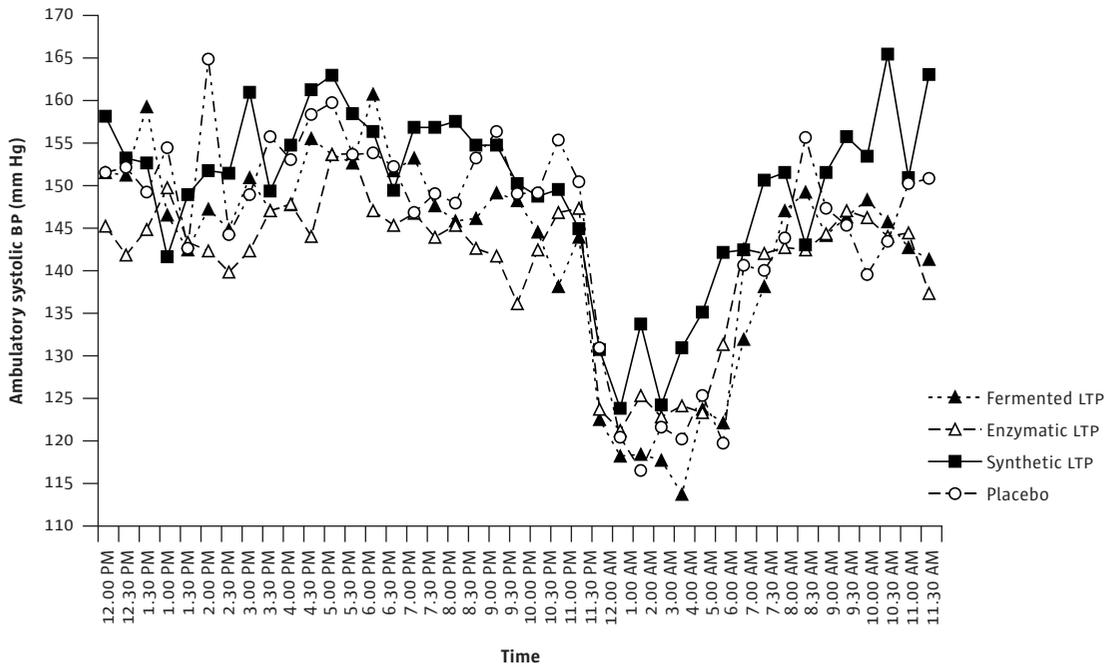
	Fermented LTP (n = 35)	Enzymatic LTP (n = 32)	Synthetic LTP (n = 34)	Placebo (n = 27)
Systolic BP, mm Hg				
Morning¹				
8-week change	-1.0 ± 1.4	-4.4 ± 1.6	-0.8 ± 1.6	-0.8 ± 2.1
Change attributed to treatment (95% CI), P-value	-0.1 (-5.7, 5.5) P = 1.00	-3.5 (-9.2, 2.1) P = 0.31	0.1 (-5.5, 5.7) P = 1.00	
Noon²				
8-week change	-3.5 ± 1.5	-2.0 ± 1.4	-1.6 ± 1.6	-1.2 ± 2.1
Change attributed to treatment (95% CI), P-value	-2.3 (-7.9, 3.3) P = 0.62	-0.8 (-6.5, 4.9) P = 0.97	-0.4 (-6.1, 5.2) P = 1.00	
Evening³				
8-week change	-5.2 ± 2.0	-5.6 ± 2.0	-3.3 ± 2.2	-3.2 ± 2.4
Change attributed to treatment (95% CI), P-value	-2.0 (-9.3, 5.4) P = 0.85	-2.4 (-9.9, 5.1) P = 0.78	-0.1 (-7.6, 7.3) P = 1.00	
Diastolic BP, mmHg				
Morning¹				
8-week change	-0.3 ± 1.0	-2.1 ± 0.9	-1.0 ± 1.3	0.1 ± 1.5
Change attributed to treatment (95% CI), P-value	-0.4 (-4.3, 3.6) P = 0.99	-2.2 (-6.3, 1.8) P = 0.40	-1.2 (-5.2, 2.8) P = 0.82	
Noon²				
8-week change	-2.2 ± 1.1	-1.5 ± 1.0	-0.6 ± 1.2	-0.4 ± 1.4
Change attributed to treatment (95% CI), P-value	-1.9 (-5.8, 2.1) P = 0.52	-1.1 (-5.1, 2.9) P = 0.85	-0.2 (-4.2, 3.7) P = 1.00	
Evening³				
8-week change	-1.6 ± 1.2	-3.0 ± 1.3	-2.2 ± 1.4	-1.3 ± 1.4
Change attributed to treatment (95% CI), P-value	-0.3 (-4.9, 4.3) P = 1.00	-1.7 (-6.4, 3.0) P = 0.73	-0.9 (-5.5, 3.8) P = 0.94	

LTP, lactotripeptides (IPP and VPP). Values are mean (±SE); two-sided P-values for the difference from placebo (Dunnett's test).

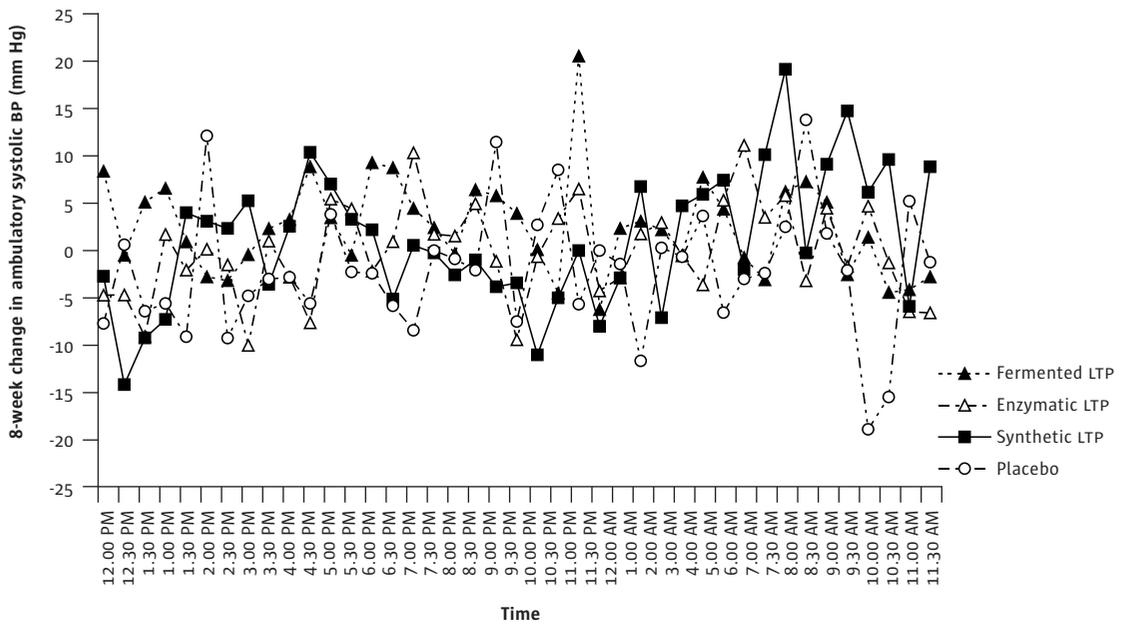
¹ Missing for 1 subjects in the placebo group.

² Missing for 3 subjects (1 enzymatic LTP, 1 synthetic LTP, 1 placebo).

³ Missing for 2 subjects (1 synthetic LTP, 1 placebo).



Supplemental figure 4.1 24-hour curves for ambulatory systolic blood pressure at week 8 in a subgroup of 58 randomized subjects in the three treatment groups and the placebo group. LTP, lactotripeptides (IPP and VPP)



Supplemental figure 4.2 24-hour curves for the 8-week change in systolic blood pressure in a subgroup of 58 randomized subjects in the three treatment groups and placebo group. LTP, lactotripeptides (IPP and VPP)



Chapter 5

A very high intake of conjugated linoleic acid, a natural *trans* fat from milk and meat, does not affect blood pressure in normotensive human subjects

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Abstract

Background: *Cis-9, trans-11* conjugated linoleic acid (CLA) is a natural *trans* fatty acid that is largely restricted to ruminant fats and is consumed in foods and supplements. Its role in blood pressure regulation is still unclear. Therefore, our objective was to study the effect of *cis-9, trans-11* CLA on blood pressure compared to oleic acid.

Methods: Sixty-one healthy men ($n = 25$) and women ($n = 36$) were sequentially fed each of 3 diets for 3 weeks, in random order, for a total of 9 weeks. Diets were identical except for 7% of energy (18.9 g in a diet of 10 MJ/day) which was provided either by oleic acid, by industrial *trans* fatty acids, or by *cis-9, trans-11* CLA. We measured blood pressure and heart rate on days 19 and 21 (period 1), days 40 and 42 (period 2) and days 61 and 63 (period 3).

Results: At baseline, mean blood pressure was 113.8 ± 14.4 mm Hg systolic and 66.3 ± 9.6 mm Hg diastolic. The effect of the CLA diet compared to the oleic acid diet was $+0.11$ mm Hg (95% confidence interval: $-1.27, 1.49$) for systolic blood pressure and -0.45 mm Hg ($-1.63, 0.73$) for diastolic blood pressure. After the industrial *trans* fatty acid diet, the blood pressure effect was $+1.13$ mm Hg ($-0.25, 2.51$) systolic and -0.44 mm Hg ($-1.62, 0.73$) diastolic compared to the oleic acid diet.

Conclusion: High intakes of *cis-9, trans-11* CLA do not affect blood pressure in healthy normotensive subjects.

Introduction

Cis-9, trans-11 conjugated linoleic acid (CLA) is a natural *trans* fatty acid that is produced in the rumens of cows, sheep and other ruminant animals through partial hydrogenation of unsaturated fatty acids from the feed by bacteria. It can also be formed from vaccenic acid in animals and in humans¹. It is consumed by humans in foods and as supplements. In general, supplements consist of 50:50 mixtures of the isomers *cis-9, trans-11* CLA and *trans-10, cis-12* CLA or mixtures of more isomers, while CLA in dairy products consists of over 90% of the *cis-9, trans-11* CLA isomer.

CLA has attracted much interest since favorable effects on health, e.g. weight reduction, improved insulin sensitivity and improved blood lipid profiles have been reported in animal studies². In addition, CLA was found to lower blood pressure in several rat models³⁻⁵. On the other hand, CLA is a *trans* fatty acid and harmful effects in the cardiovascular system cannot be excluded. Little is known about the effect of CLA on human blood pressure. To our best knowledge, only a few blood pressure trials have been done with different mixtures of CLA, all of which had a parallel controlled design. Raff et al. examined the effect of a 5-week diet supplemented with 4.7 gram of a 50:50 mixture of *cis-9, trans-11* CLA and *trans-10, cis-12* CLA, or a diet low in CLA, on blood pressure and isobaric arterial elasticity in 60 healthy, non-hypertensive young men⁶. Systolic blood pressure non-significantly increased by 3 mm Hg in the CLA group compared to the control group (p-value: 0.07), whereas diastolic blood pressure did not change. Laso et al. studied effects of a 12-week daily intervention with 3 grams of a mixture of multiple CLA isomers or placebo in 60 overweight and obese individuals with a mean blood pressure of 147/84 mm Hg and found no effect on blood pressure⁷. Additionally, Iwata et al. did a 12-week trial with a daily intake of 3.4 gram CLA mixture in 60 healthy overweight Japanese male volunteers and also found no effect of CLA on blood pressure⁸.

We conducted a randomized cross-over study to examine the effect of a high dose of *cis-9, trans-11* CLA, from an 80:20 mixture, on blood pressure in 61 normotensive healthy Dutch subjects. The blood pressure effect of CLA was compared to that of industrial *trans* fatty acids and oleic acid, and dietary intakes were fully controlled during the 9-week intervention period.

Subjects and Methods

The present study formed part of a larger trial that was set up to examine the effect of *cis-9, trans-11* CLA on cardiovascular health (Wanders et al. submitted for publication). Here, we describe the effect of *cis-9, trans-11* CLA on blood pressure, which was a pre-specified secondary endpoint of the study.

Subjects

The Medical Ethics Committee of Wageningen University approved the study. We recruited men and women aged 18 to 65 y from the Dutch population (Wageningen area) through advertisements. Potential subjects gave written informed consent before screening. We excluded subjects with glycosuria or proteinuria in morning urine samples, and with concentrations of total cholesterol >6.5 mmol/L or triglycerides >2.3 mmol/L. Subjects were also excluded if they suffered from diabetes mellitus or cardiovascular diseases, if they used cholesterol-lowering or anti-hypertensive medication, had unusual dietary habits including high alcohol intakes, had a BMI >30 kg/m², or were pregnant or lactating.

Design and randomization procedure

We performed a randomized multiple cross-over controlled feeding trial with three consecutive periods lasting three weeks each. The trial ran for 9 weeks in total, from September 25 to November 27, 2007. Before a 2-day run-in period, we randomly assigned subjects to one of the six possible diet sequences by computer-generated numbers. We performed blood pressure measurements and drew blood samples during a pre-trial screening visit, on two days at the end of each intervention period, and 3 weeks after the end of the study.

Diets and study procedures

All subjects received diets with the same composition but in different amounts depending on their energy requirement. On week days during lunch time, subjects consumed a hot meal at the Division of Human Nutrition of Wageningen University. All other food was provided daily to take away for consumption at home. Foods for the weekend and instructions for its preparation were provided each Friday. The supplied foods provided 90% of each subject's energy requirement. For the remaining 10% of energy, subjects had to select foods from a list of foods and drinks that were low in fat. Subjects recorded the selected foods daily in a diary, as well as any deviations from the diet, illnesses, and use of medication. Subjects were asked to maintain their usual lifestyle and were not allowed to drink fortified fruit juices, unfiltered coffee (containing cafestol), or to eat more than 10 pieces of liquorices a day, as the latter may affect blood pressure. We weighed subjects twice a week and adjusted energy intake when necessary in order to maintain stable body weight.

Experimental fats

The diets were intended to contain the same nutrients except for 7% of total energy, which came from special oils and fats containing *cis*-9, *trans*-11 CLA, industrial *trans* fatty acids or oleic acid. CLA was provided by a CLA-rich oil (donated by Lipid Nutrition, Wormerveer, the Netherlands). Margarines and yogurt drinks enriched with the special

oils and fats according to our specifications were manufactured by NIZO Food Research (Ede, the Netherlands). The fat in the oleic acid margarine consisted of 82% high-oleic sunflower oil (Aldoc BV, Schiedam, the Netherlands) and 18% of a standard hard stock, an interesterified mixture of palm oil and palm kernel fat (Unilever Research & Development, Vlaardingen, the Netherlands). The fat in the CLA margarine contained 25% CLA-rich oil, 10% sunflower oil, 47% high-oleic sunflower oil and 18% standard hard stock. The fat of the industrial *trans* margarine consisted of 65% partially hydrogenated vegetable fat (Melano, FUJI Oil Europe, Ghent, Belgium), 10% sunflower oil and 25% high-oleic sunflower oil. In addition, yogurt drinks were produced by enriching fat-free yogurt with 5 grams of high oleic sunflower oil, 5 grams of *cis*-9, *trans*-11 CLA-rich oil or 5 grams of partially hydrogenated vegetable fat per 100 mL, respectively. The margarines were used as a spread, and as an ingredient in bread, cookies, sauces, and gravies. Other food items were bought commercially. Subjects were not informed about the type of diet they consumed until completion of data analysis.

Diet composition

Duplicate diets were analyzed for protein, dry matter, ash, dietary fiber and digestible carbohydrates⁹⁻¹¹. CLA isomers were separated by gas chromatography on a Sil-88 column and other fatty acids on a WAX-58 column (Varian, Middelburg, the Netherlands). The composition of the free-choice items was calculated with use of Dutch food composition tables (NEVO, 2006) and included in the calculation of the average daily intake of nutrients.

Blood pressure measurements

Four trained staff members measured blood pressures at the study center during a pre-trial screening visit, on days 19 and 21 (period 1), days 40 and 42 (period 2), days 61 and 63 (period 3), and 21 days after the end of the study. After a 10-minute rest, 3 blood pressure measurements with 2 minute intervals were performed on the dominant arm in sitting position, using a validated automatic blood pressure device (Omron HEM-907) with an appropriately sized cuff. The first measurement was discarded and the subsequent two measurements were averaged. Measurements were repeated after 2 days and values of both occasions were averaged. All blood pressure measurements were performed between 7 and 9:30 AM at a fixed time using the same device by the same staff member for each individual. Subjects remained blinded toward all blood pressure values until data-analysis was completed.

Other measurements

Blood samples were taken during the pre-trial screening visit and during the intervention on the same occasions when blood pressure measurements took place. Venous blood was collected by nurses after an overnight fast, and all samples were taken at the same

time of the day to minimize within-subject variation. The blood samples were directly processed and stored at -80°C . The samples were analyzed for blood lipoproteins, as reported elsewhere (Wanders et al., submitted for publication).

Compliance to the diet was assessed by checking the diaries and by examining the fatty acid composition of cholesterylesters in plasma. The fatty acids in plasma cholesterylesters were analyzed as described previously¹². The results were combined and expressed as a proportion by weight of all fatty acids detected.

Statistical analysis

Double data entry was performed and discrepancies were solved. Treatment codes were broken after blind data analysis.

Values reported in text and tables are means with standard deviations (SD) or 95% confidence intervals (95% CI), unless stated otherwise. Response to treatment was defined as the difference in systolic blood pressure between the diets. Data were analyzed using the linear mixed model where homogeneous Compound Symmetry (CS) was selected as the covariance structure. Changes in blood pressure values were tested for treatment-, period- and carry-over effect; the latter one was tested by introducing a period by treatment interaction term. The LSD (Least Significant Difference) test was used for pairwise comparisons.

All analyses were performed with SPSS version 15.0 (SPSS, Chicago, Illinois, United States). Differences were considered statistically significant when $p < 0.05$ (two-sided).

Table 5.1 Baseline characteristics of 61 healthy Dutch subjects who completed the study on CLA and blood pressure

Characteristic	
Age, y	30.9 ± 13.7
Sex, n (% male)	25 (41)
Current smokers, n (%)	4 (6.6)
BMI, kg/m ²	22.8 ± 3.2
Total serum cholesterol, mmol/L	4.54 ± 0.77
Systolic blood pressure, mm Hg	113.8 ± 14.4
Diastolic blood pressure, mm Hg	66.2 ± 9.6
Heart rate, bpm	73.3 ± 12.4

Values are presented as means ± SD or numbers (percentages).

Results

Subjects

We enrolled 63 subjects in the trial. Two subjects withdrew from the trial; one man after 6 days because of personal reasons and one woman after 20 days due to illness, both not related to the study. **Figure 5.1** shows the number of subjects screened, excluded and randomized. The screening characteristics of the subjects who completed the study are shown in **Table 5.1**.

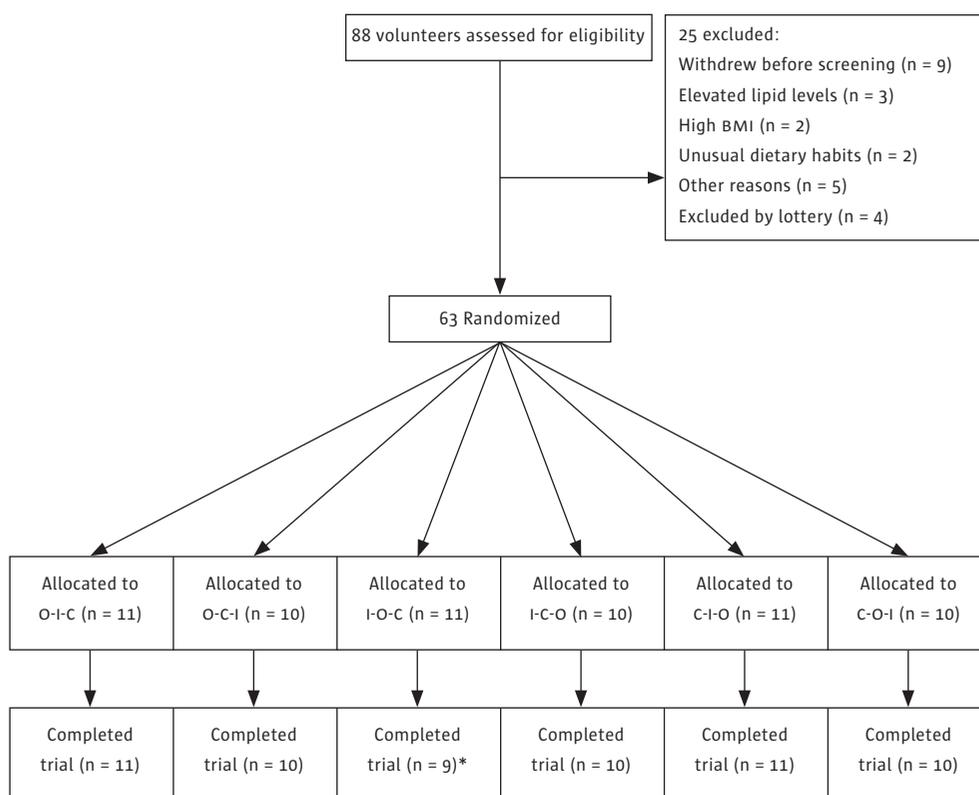


Figure 5.1 Flow chart of a randomized multiple cross-over trial of *cis*-9, *trans*-11 CLA and blood pressure in 61 normotensive Dutch subjects. Each intervention period lasted 3 weeks.

CLA indicates Conjugated Linoleic Acid; O, Oleic acid diet; I, Industrial *trans* fatty acids diet; C, *cis*-9, *trans*-11 CLA diet.

* 2 subjects discontinued the intervention due to personal reasons (n = 1) and illness (n = 1) unrelated to the trial.

These subjects were not included in the analysis.

Diets and dietary adherence

Mean daily intakes of energy and nutrients according to chemical analysis of the three experimental diets plus calculations from free-choice items are presented in **Table 5.2**. Intake of total energy, protein and carbohydrates were similar on all three diets, as were

Table 5.2 Mean daily intakes of energy and nutrients per diet¹

	Oleic acid diet	Industrial trans diet	<i>cis-9, trans-11</i> CLA diet
Energy intake, MJ/d	10.6	10.8	10.7
Energy intake, Kcal/d	2532	2568	2553
Fat, % of energy	39.7	40.1	39.7
Saturated fatty acids	10.5	13.8	11.3
<i>C12:0 (lauric)</i>	0.7	0.2	0.8
<i>C14:0 (myristic)</i>	0.9	0.7	1.1
<i>C16:0 (palmitic)</i>	5.7	9.7	6.3
<i>C18:0 (stearic)</i>	2.0	2.3	1.9
Total <i>cis</i> fatty acids	27.4	17.1	17.7
<i>Cis-9 C18:1 (oleic)</i>	23.1	11.3	13.4
<i>Cis-9, cis-12 C18:2 (linoleic)</i>	3.2	4.1	3.4
Total <i>trans</i> fatty acids	0.2	7.5	9.1
Total <i>trans C18:1</i>	<0.1	7.3	<0.1
<i>Trans-9 C18:1 (elaidic)</i>	<0.1	3.1	<0.1
<i>Trans-10 C18:1</i>	<0.1	1.5	<0.1
<i>Trans-11 C18:1 (vaccenic)</i>	<0.1	0.8	<0.1
Total CLA	0.1	0.1	9.0
<i>Cis-9, trans-11 CLA</i>	0.1	0.1	6.9
<i>Trans-10, cis-12 CLA</i>	0.0	0.0	1.5
Protein, % of energy	12.6	11.8	12.8
Carbohydrates, % of energy	46.3	46.7	46.0
Alcohol, % of energy	1.4	1.4	1.5
Dietary fiber, g/MJ	3.0	2.9	2.9
Cholesterol, mg/MJ	22.3	22.8	21.9

CLA indicates Conjugated Linoleic Acid.

¹ According to chemical analysis of duplicates of the diets (based on 3 samples per diet); contributions from free-choice items were included as described in Methods.

intake of total fat, dietary fiber and alcohol. The cholesterylesters of all subjects showed that the CLA proportion was 3.1 times higher after the *cis-9, trans-11* CLA diet than after the oleic acid diet ($p < 0.001$).

Diaries kept by the subjects revealed only minor deviations from the protocol. Six subjects reported nausea for 1 or 2 days on the *cis-9, trans-11* CLA and industrial *trans* fatty acids diets, and 2 while on the oleic acid diet. Three subjects reported diarrhea for 1-2 days while on the *cis-9, trans-11* CLA diet.

Blood pressure

At baseline, mean blood pressure was 113.8 ± 14.4 mm Hg systolic and 66.3 ± 9.6 mm Hg diastolic. **Table 5.3** presents the average blood pressure and heart rate at the end of each dietary intervention period. We observed no significant differences in blood

pressure or heart rate among the diets. The effect on blood pressure of the *cis-9, trans-11* CLA diet compared to the oleic acid diet was +0.11 (-1.27, 1.49) mm Hg systolic and -0.45 (-1.63, 0.73) mm Hg diastolic. After the industrial *trans* fatty acids diet, the treatment effect compared to the oleic acid diet was +1.13 (-0.25, 2.51) mm Hg for systolic blood pressure and -0.44 (-1.62, 0.73) mm Hg for diastolic blood pressure. We observed a significant period effect for blood pressure; in the group as a whole systolic blood pressure increased by 2.38 (1.00, 3.75) mm Hg ($p < 0.001$). We found no carry-over effect for systolic or diastolic blood pressure ($p = 0.16$ and $p = 0.91$, respectively).

Table 5.3 Blood pressure and heart rate at the end of the three intervention periods in 61 healthy men and women, by treatment

	Oleic acid diet	Industrial trans diet	<i>cis-9, trans-11</i> CLA diet
Systolic blood pressure, mm Hg	113.3 (110.5, 116.1)	112.1 (110.3, 116.0)	113.2 (109.3, 115.0)
Diastolic blood pressure, mm Hg	65.3 (63.3, 67.3)	65.7 (63.7, 67.7)	65.7 (63.7, 67.7)
Heart rate, bpm	70.4 (67.2, 73.6)	70.7 (67.5, 73.9)	70.3 (67.2, 73.5)

CLA indicates Conjugated Linoleic Acid. Values are mean (95% CI); data were analyzed using the linear mixed model where homogeneous Compound Symmetry (CS) was selected as covariance structure. No significant differences among the diets were found.

Other measurements

Body weight did not differ among the three diets. Mean body weight (\pm SD) was 70.1 \pm 12.2 kg on the oleic acid diet, 70.1 \pm 12.2 kg on the industrial *trans* fatty acids diet and 70.1 \pm 12.0 kg on the *cis-9, trans-11* CLA diet. However, over 9-weeks intervention period the average body weight of the total group decreased on by 0.6 \pm 1.7 kg ($p = 0.006$).

Discussion

In the present study among 61 healthy normotensive subjects aged between 18 and 65 y, we found no effect of *cis-9, trans-11* CLA on blood pressure or heart rate. Also, industrial *trans* fatty acids did not affect blood pressure or heart rate.

Strengths of the study were its randomized cross-over design and strictly controlled food intake, which was confirmed by the fatty acid composition of plasma cholesterylesters. We used a strict and standardized protocol for blood pressure measurement (e.g. rest, validated device, appropriately sized cuff, blinding). Body weight, an important blood pressure determinant, was carefully monitored and was similar for the three diets.

The present trial was not primarily designed to investigate effects on blood pressure, and could therefore have some limitations. Sample size was calculated to be able to

detect an effect on LDL-cholesterol, the primary outcome of the study. Nevertheless, power was sufficient to detect small changes in blood pressure: we could have detected a difference in systolic blood pressure of 2 mm Hg with a power of 80%. Second, although food intake was controlled, subjects were free to use coffee and table salt. However, due to the cross-over design the effect of coffee and table salt use is likely to be canceled out. Furthermore, it should be noted that blinding of treatment was not completely successful. None of the subjects recognized the difference between the *cis*-9, *trans*-11 CLA and the oleic acid diet, but almost all (96%) subjects recognized the industrial *trans* fatty acids diet due to the solid margarine. Yet, as blood pressure was a secondary outcome and most subjects were unaware of this aim of the study, it is unlikely that awareness of order of the diets has impacted our blood pressure values. Another limitation is the relatively short duration of the dietary intervention periods (3 weeks each). Significant blood pressure changes with dietary measures, however, have been achieved within such a short time period, including the well-known DASH study where most of the treatment effect was already achieved after 2 weeks of dietary intervention¹³. Remarkably, blood pressure of the group as a whole increased slightly but significantly during the 9-week trial (2.4 mm Hg, $p < 0.001$). This is in contrast to what is often observed in blood pressure trials. Because blood pressure was a secondary outcome, subjects were not focused on lowering their blood pressure. Intentional lifestyle or behavioral changes are therefore unlikely and we cannot explain this observation. However, because all subjects received the treatments in random order we do not think this has influenced the overall outcome of our study. Finally, our subjects were young, lean and healthy, and the effects we found in our study may not apply to individuals with higher blood pressure levels, e.g. elderly, obese individuals and patients with hypertension.

Up until now, *trans* fatty acids have not been clearly associated with blood pressure¹⁴⁻¹⁷. However, most previous studies have focused on industrial *trans* fatty acids¹⁴⁻¹⁷ or CLA mixtures with a high content of *trans*-10, *cis*-12 CLA⁶⁻⁸. The *trans*-10, *cis*-12 isomer has been suggested to have more unfavorable effects on cardiovascular health than *cis*-9, *trans*-11 CLA^{2,18}. Less was known about the effect of *cis*-9, *trans*-11 CLA. Although different effects of *cis*-9, *trans*-11 CLA on blood pressure might have been expected, the results from our study are in line with previous studies on the effects of industrial *trans* fatty acids and CLA mixtures on blood pressure.

Thus, we conclude that the intake of *cis*-9, *trans*-11 CLA, exclusively found in dairy products, does not affect blood pressure in healthy normotensive human subjects.

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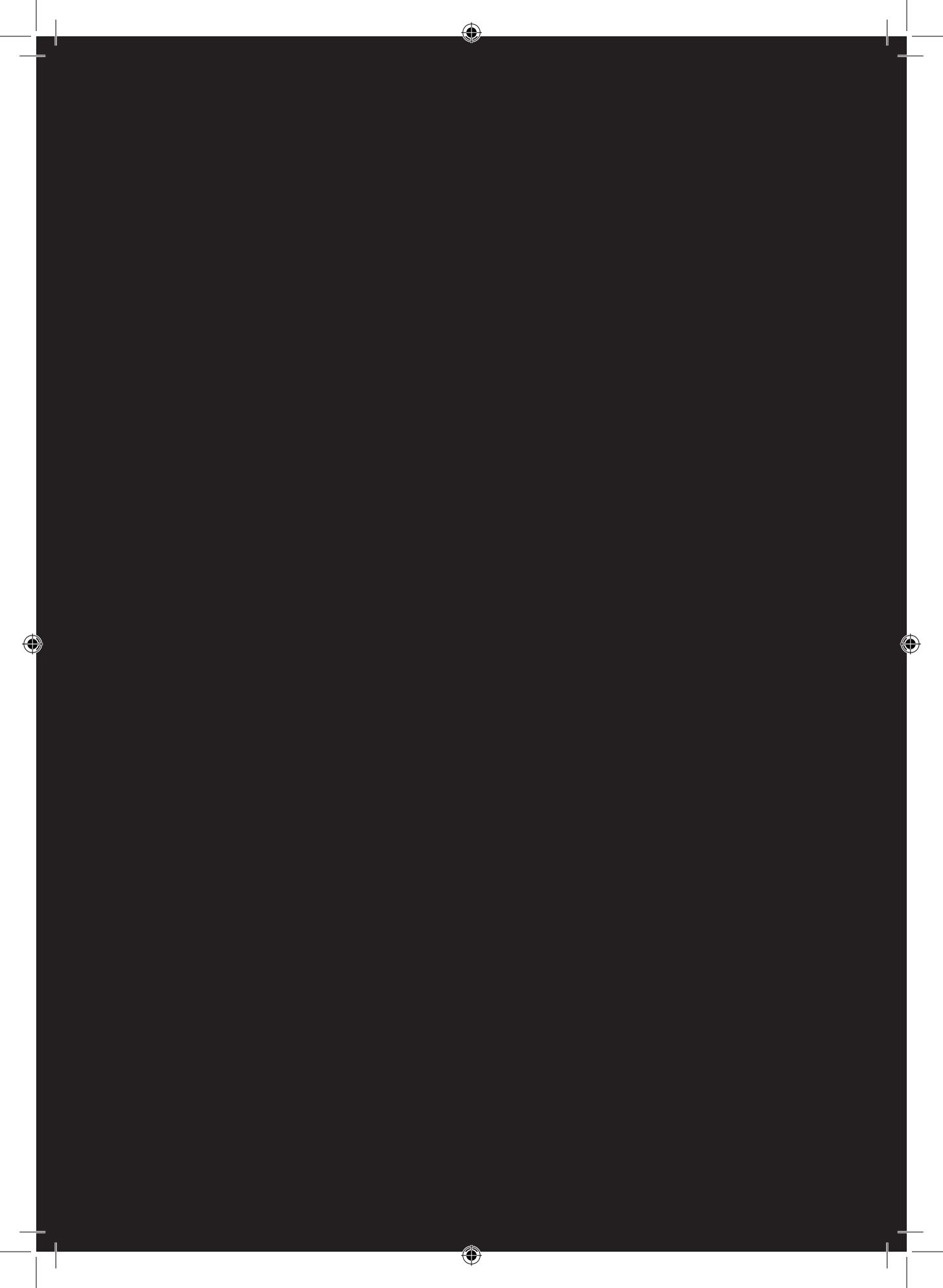
Conflict of interest

None

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Chapter 6

General discussion

The worldwide prevalence of hypertension is increasing rapidly¹, which calls for effective public health measures. Whether intake of dairy or specific dairy foods could play a role in reducing population blood pressure remains to be established. The research described in this thesis comprises observational studies and intervention studies. The main objectives were [1] to examine the association of (specific types of) dairy intake with blood pressure level and incident hypertension in the Netherlands, a country with a traditionally high dairy intake, and [2] to assess the blood pressure effect of two dairy components that have recently attracted much attention, i.e. lactotripeptides (IPP and VPP) and *cis*-9, *trans*-11 conjugated linoleic acid (CLA). In this chapter the main findings, methodological issues and the validity of the studies are discussed for observational studies (**chapters 2 and 3**) and trials (**chapters 4 and 5**), respectively. This is followed by a review of proposed biological mechanisms that may explain an effect of dairy intake on blood pressure. Finally, the evidence provided in this thesis is put into public health perspective and suggestions for further research are given.

Main findings

The findings described in this thesis are presented in **Table 6.1**. Blood pressure level showed no consistent association with overall dairy intake or intake of specific dairy foods in a cross-sectional analysis that was conducted in a general population of 21,553 Dutch subjects (MORGEN study, **chapter 2**). In longitudinal analysis, however, inverse relations of low-fat dairy with incident hypertension were observed, both in 3454 young and middle-aged subjects (subcohort of the MORGEN study, **chapter 2**) and in 2245 older subjects (Rotterdam study, **chapter 3**). In both studies, the risk of hypertension was reduced by ~20% in subjects who consumed more than 150 mL (~1 serving) of low-fat dairy per day. Other dairy foods, i.e. fermented dairy, high-fat dairy, milk and milk products, and cheese were not consistently associated with risk of hypertension (**chapters 2 and 3**). When focusing on specific dairy components, we found no blood pressure effect for lactotripeptides (i.e. IPP and VPP) in an 8-week randomized controlled trial in 135 middle-aged Dutch subjects with untreated elevated blood pressure (**chapter 4**). Blood pressure was also not affected by high intakes of *cis*-9, *trans*-11 CLA in a 9-week randomized cross-over trial in 61 normotensive young Dutch subjects (**chapter 5**).

Dairy food intake in relation to blood pressure: methodological issues

A major part of this thesis is based on observational studies that are subject to certain limitations. Methodological issues of the MORGEN study and the Rotterdam study have been discussed in the specific chapters (**chapters 2 and 3**). Some of these aspects are highlighted here because of their relevance for the validity of the findings.

Table 6.1 Main findings of the studies described in this thesis

Dairy intake and blood pressure or hypertension		
Design	Exposure	Main results
<p>Cohort: MORGEN study Type: cross-sectional Population: 21,553 Dutch adults aged 20-65y Endpoint: BP level</p> <p>(Ch2)</p>	Intake of total, low-fat, high-fat, and fermented dairy, milk and milk products, yogurt, and cheese.	No consistent relation between overall dairy or intake of specific dairy foods with BP level.
<p>Cohort: subcohort of MORGEN study Type: prospective Population: 3454 Dutch adults aged 20-65y Endpoint: 5y incidence of HT</p> <p>(Ch2)</p>	Intake of total, low-fat, high-fat, and fermented dairy, milk and milk products, yogurt, and cheese.	<p>Overall: Overall dairy, specific dairy groups and dairy products were not clearly related to risk of developing HT. Low-fat dairy: Multivariate OR (95% CI): 1.00, 0.78 (0.61, 1.00), 0.81 (0.63, 1.03) and 0.82 (0.64, 1.06, P-trend: 0.24) in consecutive quartiles of intake.</p>
<p>Cohort: ROTTERDAM study Type: prospective Population: 2245 Dutch adults aged \geq55y Endpoint: 2y and 6y incidence of HT</p> <p>(Ch3)</p>	Intake of total, low-fat, high-fat, and fermented dairy, milk and milk products, and cheese.	<p>Overall (2y): Total dairy, milk and milk products were inversely associated with HT. Fermented dairy tended to be inversely related to risk of HT. No clear association of high-fat dairy and cheese with HT. Low-fat dairy (2y): Multivariate HR (95% CI): 1.00, 0.75 (0.60, 0.92), 0.77 (0.63, 0.96) and 0.69 (0.56, 0.86, P-trend: 0.003) in consecutive quartiles of intake.</p> <p>Overall (6y): Total dairy, milk and milk products were inversely associated with HT. No clear association of fermented dairy, high-fat dairy and cheese with HT. Low-fat dairy (6y): Multivariate HR (95% CI): 1.00, 0.86 (0.72, 1.04), 0.81 (0.67, 0.97) and 0.84 (0.70, 1.01, P-trend: 0.07) in consecutive quartiles of intake.</p>
Dairy components		
<p>Type: Parallel RCT Population: 135 adults aged 35-70y with untreated elevated BP Intervention: 8-wk intervention: 200 mL dairy drink with 14 mg lactotripeptides or placebo</p> <p>(Ch4)</p>	Lactotripeptides (IPP and VPP) obtained by fermentation, enzymatic hydrolysis, or chemical synthesis.	Intervention with lactotripeptides did not affect BP ($p = 0.46$). The mean difference (95% CI) in SBP response between the treatment and control group: 2.8 mm Hg (-2.6, 8.2) for fermented lactotripeptides, -0.5 mm Hg (-6.0, 5.0) for enzymatic lactotripeptides, 1.6 mm Hg (-3.9, 6.9) for synthetic lactotripeptides.
<p>Type: Multiple cross-over RCT Population: 61 normotensive adults aged 18-60y Intervention: 3 diets for 3 weeks: 7en% provided by oleic acid, industrial <i>trans</i> fatty acid or CLA</p> <p>(Ch5)</p>	<i>Cis-9, trans-11</i> conjugated linoleic acid (CLA)	High intake of <i>cis-9, trans-11</i> CLA did not affect BP. SBP changed -0.1 mm Hg (-1.49, 1.27) compared to control treatment (oleic acid) ($p = 0.87$). Also industrial <i>trans</i> fatty acids did not affect BP.

Study design

The association between dairy intake and blood pressure was examined in the baseline dataset of the MORGEN study, using cross-sectional data (**chapter 2**). A main drawback of this design is that dairy intake and blood pressure were assessed at the same moment in time which makes it difficult to address the temporality of the association. It is possible that subjects with elevated blood pressure, or otherwise at increased cardiovascular risk, changed their overall dairy intake or specific dairy foods (e.g. from high-fat to low-fat dairy) upon medical advice. For this reason, we excluded subjects with clinically diagnosed hypertension (i.e. using antihypertensive medication) from the analysis. Part of the remaining subjects, however, had untreated (pre)hypertension. We consider intentional dietary changes in this group unlikely since elevated blood pressure is often asymptomatic and advice on dairy products is not common practice in the Netherlands. Apart from the cross-sectional analysis, we also examined the association between dairy intake and incident hypertension using prospective data of the MORGEN study (**chapter 2**) and the Rotterdam study (**chapter 3**). In these studies dairy intake was assessed before the diagnosis of hypertension and misinterpretation of findings due to (intentional) dietary changes is therefore not an issue.

The ~20% reduced risk for hypertension that we found in the prospective analyses (**chapters 2 and 3**) suggest that low-fat dairy could lower blood pressure. The cross-sectional data of the MORGEN study, however, were not in line with this finding, since there was no evidence for an association of low-fat dairy intake with blood pressure levels (**chapter 2**). We have no ready explanation for this discrepancy. Apart from chance, which can never be excluded in epidemiological studies, it could be speculated that the somewhat older age of the prospective cohorts played a role. The cardiovascular system becomes less resilient during ageing, and the sensitivity of blood pressure to dietary influences, including dairy intake, may have increased. It is also possible that subjects who reported low-fat dairy intake during the baseline survey of the MORGEN study actually had a less favorable intake of dairy products in the past (see **chapter 1, Figure 1.1**). The baseline blood pressure level, which can be considered a cumulative endpoint of past exposure, may still reflect the intake of e.g. high-fat dairy intake earlier in life. Changes in the other direction (i.e., from low-fat to high-fat dairy) during the subsequent follow-up period are less likely to have occurred, and the associations with incident hypertension are probably not distorted by such dietary changes. Finally, there could be methodological reasons why prospective and cross-sectional data are in disagreement. In a cross-sectional analysis, one attempts to explain between-subject variation, whereas in longitudinal analysis the temporal change within a subject is the outcome of interest. In case of the latter, much of the within-subject variation (e.g. due to genetic factors) is eliminated in the statistical analysis, leaving more room for the detection of weak associations with dietary or lifestyle factors (in this case low-fat dairy intake).

Assessment of dairy intake

In our observational studies (**chapters 2 and 3**) dairy intake was assessed by a food frequency questionnaire that inquired about the consumption of foods and beverages over the past year. This is a generally accepted tool for the assessment of habitual diet in large-scale epidemiological studies. The 178-item food frequency questionnaire of the MORGEN study (**chapter 2**) was validated against 12 monthly 24-hour recalls over a 1-year period and showed a good correlation ($r = 0.7-0.8$) for milk and milk products². The 170-item food frequency questionnaire used in the Rotterdam study (**chapter 3**) was validated against a 2-week food diary showing a correlation of 0.7 for calcium³. In the Netherlands, about 75% of the dairy products are consumed during meals, i.e. at fixed moments during the day⁴, which may have helped participants in recalling their dairy intake. Although the absolute amount cannot be assessed accurately by food frequency questionnaires, we assume that subjects have been adequately ranked for their dairy intake. Some random misclassification of dairy intake, however, is inevitable. This would have weakened our associations between dairy intake and hypertension, meaning that the observed 20% risk reduction for hypertension with intake of low-fat dairy could be an underestimation of the true association. We can also not exclude the possibility that weak associations of overall dairy or specific types of dairy with blood pressure or hypertension have been missed due to misclassification of intake.

Differential misclassification of dairy intake is a larger threat to the validity of epidemiological studies. Obese individuals in our studies may have underreported total food intake, which will also apply to dairy foods. For this reason, we adjusted dairy intake for total energy intake according to the residual method⁵, before dividing the study population into quartiles. By expressing dairy intake relative to a subject's energy intake differential misclassification due to underreporting of total food intake was probably eliminated to a large extent. Nevertheless, we cannot ignore the fact that obese subjects may have differentially underreported (e.g. specifically for high-fat dairy products). It should be noted, however, that less than 10% of the participants from the MORGEN study (**chapter 2**) and the Rotterdam study (**chapter 3**) was obese. Should differential misclassification have occurred, the influence on the outcome of our study is likely to be small.

Assessment of blood pressure and hypertension

An individual's blood pressure varies in different seasons, from day to day, and also during the day. Other factors, such as room temperature, blood pressure device, cuff size, posture, and a person's mental and physical state also influence blood pressure. In the MORGEN study (**chapter 2**) and the Rotterdam study (**chapter 3**) blood pressure at baseline and during follow-up visits was measured twice at each occasion, with a random-zero sphygmomanometer, and the average value was used in the analyses. It is

likely that random error occurred in the assessment of blood pressure. This may have led to imprecision resulting in wider 95% confidence intervals for the association of dairy intake with incident hypertension, but it is unlikely to have influenced the strength of the association itself. Multiple blood pressure measurements taken at different occasions over a longer period of time is the only way to reduce random error, but this was not feasible in these large population-based cohort studies.

In our prospective analyses we used incident hypertension as the primary outcome, defined as blood pressure $\geq 140/90$ mm Hg or use of antihypertensive medication. Using this arbitrary and robust cut-off point has a major disadvantage; much attention is paid to small changes around the cut-off point, while changes elsewhere in the distribution are ignored. In this way, part of the effect of dairy intake on blood pressure will be missed. In other words, the effect of dairy in the pre-hypertensive range, which is particularly relevant for primary prevention, remains unknown. Although we lost information by dichotomizing blood pressure, we preferred studying incident hypertension to blood pressure changes during follow-up to be able to include subjects who initiated antihypertensive medication during follow-up in the analysis. In treated subjects actual blood pressure levels are obviously influenced by drug use which may overrule possible effects of diet.

Confounding & effect modification

It could be argued that the lower incidence of hypertension in subjects who used low-fat dairy products may not be attributable to a specific effect of these foods but to other aspects of an overall healthier lifestyle that are not (fully) captured by the collected information. Important blood pressure determinants for which the analyses were not fully controlled included physical activity and the intake of sodium and potassium from non-dairy sources. Although we controlled for several foods rich in potassium (e.g. bread, fruits, and vegetables), no adjustment was made for whole-grain foods. With regard to sodium intake, we adjusted our analyses for bread and meat, but not for use of table salt or other salt-rich foods such as soup or pizza. In addition, we had incomplete data on physical activity in both cohort studies. In the MORGEN study (**chapter 2**), data on physical activity were available for only 77% of the cohort. Repeating multivariate analysis with additional adjustment for physical activity in subjects with complete data, however, did not materially influence the associations of dairy intake with blood pressure or hypertension. In the Rotterdam study (**chapter 3**), data on physical activity during follow-up have only recently become available for 38% of the subjects that were included in our analysis. Physical activity expressed as MET-scores in this subgroup was on average 106 hours per week and did not vary with dairy intake; 102, 109, 108, and 103 hours per week in consecutive categories of dairy intake, respectively (P-trend: 0.40). Apart from this, it should be noted that in both our

observational studies the lowest category of energy-adjusted dairy, which was used as reference, included more men which resulted in higher intakes of total energy, meat, bread, and total and saturated fat than in other categories. We performed additional analyses stratified by sex, and observed an inverse association between low-fat dairy and hypertension both within men and women (**chapters 2 and 3**). In both observational studies the associations of dairy with hypertension appeared to be robust and adjustment for many potential confounders did not change the risk estimates.

We adjusted our analyses for BMI because it is an important blood pressure determinant and could act as a confounder, as described previously. We also checked whether BMI should be regarded as an effect modifier. In the MORGEN study (**chapter 2**) the association between low-fat dairy and incident hypertension did not vary by overweight status, but in the Rotterdam study (**chapter 3**) the association was more pronounced in overweight individuals. It could be that overweight at older age puts more pressure on the cardiovascular system, although this finding could also be due to chance. Finally, as dairy intake has been related to weight management and BMI⁶, body weight could also be an intermediate factor. We therefore ran the statistical models with and without adjustment for BMI which did not materially affect the risk estimates. Moreover, in the prospective part of the MORGEN study (**chapter 2**) weight gain was on average 1.5 kg and did not vary across categories of dairy intake. In the Rotterdam study (**chapter 3**) additional adjustment for change in body weight did not attenuate the observed association between low-fat dairy and risk of hypertension. It is therefore unlikely that body weight was an intermediate factor in the association of low-fat dairy with risk of hypertension.

Interpretation and external validity of the observational findings

The association of dairy intake with blood pressure and hypertension has been examined in several populations in Europe (The Netherlands, Spain, France), Iran, and the US, as summarized in **chapter 1 (Table 1.2.)**. These studies have been performed in children⁷, young adults⁸⁻¹⁰, middle-aged adults¹¹⁻¹⁵, and older adults¹⁶⁻¹⁸. The average total dairy intake of young and middle-aged adults from the MORGEN study (**chapter 2**) and older adults from the Rotterdam Study (**chapter 3**) was 350-400 mL (~2.5 servings), ranging from less than 1 serving/d in the lowest category to ~5 servings/d in the highest category of dairy intake. In another Dutch cohort of 2064 men and women aged 50-70 y (Hoorn study), the average total dairy intake was somewhat higher (~4 servings/d)¹⁷. The range of dairy intake in our cohort studies was more or less similar to that of other middle-aged and older populations in Spain⁸ and the US^{13, 15, 18} (i.e. 110 to 900 g/d), while the range of intake in two French populations was smaller (i.e. 80 to 400 g/d)^{12, 14}. The dataset of the MORGEN study (**chapter 2**) was sufficiently large to allow a more detailed examination of dairy and blood pressure within subjects who had the lowest intake (i.e.

bottom quintile of the distribution, range of total dairy intake 0-185 g/d), but no associations with blood pressure were found within this low range of intake.

Previous observational studies generally suggested an inverse association between dairy food intake and blood pressure or hypertension (**Table 1.2.**), but findings are not conclusive. First, beneficial effects were mainly found for low-fat rather than high-fat dairy products in some studies^{8, 15, 18} (**chapter 2** and **3**), while this was not confirmed by others^{7, 9, 16, 17}. In about half of the studies, the association of specific products (e.g. cheese, desserts, yogurt) with blood pressure or hypertension was examined^{9, 10, 14-17}, but no consistent relationships emerged from these data. Second, significant associations in some studies were only observed in subgroups, such as overweight individuals⁹ or men¹². In our own studies, we observed an inverse association between low-fat dairy and incident hypertension (**chapters 2** and **3**), whereas dairy intake appeared not to be related to blood pressure level (diastolic blood pressure decreased ~1 mm Hg, while systolic blood pressure increased ~1 mm Hg, **chapter 2**). This is in contrast to other studies where dairy intake was inversely related to systolic but not diastolic blood pressure¹³, or to blood pressure level but not to change in blood pressure^{12, 16, 17}. Finally, while previous observational studies generally observed a 'dose-response relation' between dairy intake and risk of hypertension, we found indications for a 'threshold effect'. Based on these inconsistencies, we conclude that chance or other phenomena, such as publication bias, may have played a role in this field of observational research.

Dairy components and blood pressure: methodological issues

For answering the research questions on dairy components and blood pressure, we made use of the randomized trial design. Important methodological issues, e.g. randomization, blinding, and compliance, have been discussed in the specific chapters (**chapters 4** and **5**). Here we limit our reflections to more general aspects of blood pressure trials and possible effects unrelated to the intervention.

Study population

The trial on lactotriptides (**chapter 4**) was set up to study blood pressure effects in untreated hypertensive subjects. Well-known phenomena in blood pressure research are 'white-coat hypertension' and 'regression-to-the-mean'¹. To ensure inclusion of truly hypertensive subjects and to reduce the possibility of 'regression-to-the-mean' during the intervention period, we applied strict measurement conditions in a comfortable non-clinical environment at several screening occasions over time. Indeed, blood

¹ Regression to the mean is a statistical phenomenon that can make a natural variation in repeated data look like a real change. It happens when usually large or small measurements tend to be followed by measurements that are closer to the mean¹⁹.

pressure showed a huge decline during the screening phase and over 65% of the screened subjects could not be enrolled because systolic blood pressure dropped below 140 mm Hg. Blood pressure further dropped during the 2-week run-in phase, leaving only 74 subjects (55% of those enrolled) with a systolic blood pressure above 140 mm Hg at the start of the trial. Should only truly hypertensive subjects be susceptible to a blood pressure lowering effect of lactotriptides, it could be possible that we missed an effect in our trial since only 10% of our study population had moderate to severe hypertension.

In the trial on *cis-9, trans-11 CLA* (**chapter 5**) blood pressure was a secondary outcome. Subjects using antihypertensive medication were not allowed to participate, but other exclusion criteria that may be relevant when studying blood pressure (e.g. age, BMI, initial blood pressure) were not applied. As a result, the trial was conducted in young and lean, healthy subjects with normal blood pressure levels (mean systolic blood pressure 114 mm Hg) which puts restrictions on the generalizability of the findings for CLA to other populations.

Study design

In **chapter 4**, the effect of lactotriptides was investigated in an 8-week randomized placebo-controlled trial. The trial was conducted according to Good Clinical Practice, providing quality assurance for the results. The randomization succeeded in achieving its goal; relevant subject characteristics (including blood pressure) were balanced over the treatment and control groups. The trial was conducted in a double-blind fashion, i.e. until data-analysis was completed subjects and research staff remained blinded to [a] treatment allocation and [b] all blood pressure values. For safety aspects, a research assistant who was not otherwise involved in the conduct of the trial checked whether systolic blood pressure did not increase by more than 30 mm Hg compared to a previous visit (i.e. pre-defined limit approved by Medical Ethics Committee and collaborating general practitioners). This was not the case.

Effects of *cis-9, trans-11 CLA* on blood pressure (**chapter 5**) were studied in a 9-week randomized cross-over trial with three consecutive periods lasting three weeks each. This design allows within subject comparison of the diets containing oleic acid (i.e. control), industrial *trans* fatty acids or *cis-9, trans-11 CLA*. Despite the single-blind fashion of this trial, research assistants who assessed blood pressure were unaware of treatment allocation. Subjects were not informed about their blood pressure or type of diet they consumed until completion of data analysis. Blinding of treatment, however, was not completely successful as almost all (96%) subjects recognized one of the three diets (industrial *trans* fatty acid diet) due to the solid margarine. Yet, as blood pressure was a secondary outcome and most subjects were not aware of this aim of the study, it

is unlikely that awareness of order of the diets would have had a large impact on blood pressure values.

Treatment and compliance

In our trials high doses of lactotriptides and CLA (**chapters 4 and 5**) were used that cannot be achieved by normal dairy consumption. The dose of lactotriptides which we used in our trial (**chapter 4**) was 14 mg per day. Although the amount of lactotriptides in a normal diet is unknown, our dose was higher than what was used in most previous trials on lactotriptides (2-6 mg per day). Our trial on CLA (**chapter 5**) contained about 20 grams per day (with an energy intake of 10 MJ), whereas a normal diet contains approximately 0.3 grams CLA per day^{20, 21}. Our trials, however, aimed to unravel a possible underlying mechanism for the observed associations between (low-fat) dairy and hypertension and using these high doses ensured sufficient contrast between treatments to observe relevant differences.

Another important issue is the duration of the intervention. In most dietary intervention studies, effects on blood pressure (if any) become visible within 4-8 weeks²²⁻²⁴. Hence, an 8-week intervention with a dose of 14 mg lactotriptides equivalents (**chapter 4**) should have been sufficiently long for detecting an effect on blood pressure, if present. In the multiple cross-over trial on CLA (**chapter 5**), the intervention periods were only three weeks. A longer intervention would have been more appropriate, although blood pressure changes have been achieved within such a short time period, e.g. in the DASH trial where blood pressure decreased after two weeks and was maintained for the next six weeks²².

Compliance to treatment is important in an etiological study in which one aims to assess the maximal effect of the intervention on blood pressure. In the trial on lactotriptides (**chapter 4**) compliance was assessed by checking diaries in which subjects recorded consumption and time of test products on a daily basis. Additionally, empty and full cups that were returned by subjects were counted. Compliance in this study was satisfactory; only 4 subjects consumed less than 80% of their provided test products. Moreover, only one subject dropped out in week 3 of the intervention for personal reasons unrelated to the trial. In the strictly controlled feeding trial on CLA (**chapter 5**), subjects consumed their hot meal under supervision at the research center and compliance was assessed by checking diaries and measurements of blood fatty acids. Diaries kept by the subjects revealed only minor deviations from the protocol and the CLA proportion in blood fatty acids was 3.1 times higher after the CLA diet than after the oleic acid diet. Only two subjects dropped out for personal reasons unrelated to the trial.

Assessment of blood pressure

Blood pressure is subject to variation as described previously in this chapter. In order to reduce random error as much as possible, standardized protocols (at least 10 minutes rest, validated device, appropriately sized cuff) were applied in our trial on lactotriptides as described in **chapter 4**. Blood pressure was a secondary, though prespecified, outcome in the CLA trial (**chapter 5**) and a standardized blood pressure protocol was also applied in this study. Strict measurement conditions strongly increased the precision with which blood pressure was assessed. Random error, however, can never completely be ruled out. This generally leads to wider confidence intervals for the blood pressure effect due to treatment, but is unlikely to have affected the internal validity of our studies and size of the effect estimates.

Effects unrelated to the intervention

In our trial on lactotriptides (**chapter 4**), the control group also showed favorable changes in blood pressure during the intervention period. These changes cannot be attributed to the lactotriptides and other external factors must explain these changes. First, as subjects with elevated blood pressure were selected to participate in the trial, this effect may partly be explained by ‘regression-to-the-mean’. Second, the measured effect might be the result of study participation rather than the result of the intervention. Finally, we cannot exclude the possibility that the dairy drink itself (i.e. 200 mL low-fat yogurt drink per day) influenced blood pressure. Because the trial was not set up to test this hypothesis, the blood pressure effect of the low-fat yogurt remains unknown.

In our trial on CLA (**chapter 5**), invalid conclusions due to effects unrelated to the intervention are not very likely. First, blood pressure was not an inclusion criterion in this study. Inclusion of normotensive subjects reduced the possibility of the ‘regression-to-the-mean’ phenomenon. Second, food intake was largely controlled; 90% of the foods were supplied by the university where subjects also consumed their hot meal during week days. Intentional dietary changes during the trial are therefore excluded. Third, blood pressure was a secondary outcome of the trial and most subjects were unaware of this aim of the study. Subjects were not focused on lowering their blood pressure during the trial making intentional lifestyle or behavioral changes less likely. Finally, the trial was conducted according to a randomized cross-over design meaning that subjects were their own control.

Interpretation and validity of the findings from intervention studies

Previous trials on the lactotriptides IPP and VPP were rather consistent in showing a blood pressure lowering effect (1.5 to 11.0 mm Hg systolic)^{25, 26}, mainly in Japanese and Finnish subjects. It should be noted, however, that in some placebo-controlled trials the results were reported as changes from baseline within groups instead of comparisons

of changes between groups. In addition, in several studies initial blood pressure levels, despite randomization, tended to be higher in the intervention than in the control group meaning that effects attributed to lactotriptides may actually have resulted from 'regression-to-the-mean'. In our relatively large trial relevant subject characteristics were equally balanced over the treatment and placebo groups. We used a high dose of lactotriptides, different blood pressure measurements, and we measured parameters of the proposed underlying mechanism. We did not find a blood pressure-lowering effect of the lactotriptides IPP and VPP. Although we consider it unlikely, we cannot exclude that lactotriptides may exert their effect only in subjects with clinically established hypertension. We were the first who published a study with "null" results in this field. More studies that could not confirm a beneficial effect of these two lactotriptides on blood pressure or ACE inhibition have recently been published^{27, 28}. We therefore cannot exclude publication bias in this field of research which is also suggested by the meta-analysis by Pripp et al²⁵.

Little research has been done on CLA and blood pressure. Previous studies have focused on CLA mixtures with a high content of *trans*-10, *cis*-12 CLA²⁹⁻³¹. The *trans*-10, *cis*-12 isomer has been suggested to have more unfavorable effects on health than *cis*-9, *trans*-11 CLA^{32, 33}. Less was known about the effect of *cis*-9, *trans*-11 CLA. Although different effects of *cis*-9, *trans*-11 CLA on blood pressure might have been expected, the results from our study are in line with previous studies on the effects of CLA mixtures on blood pressure²⁹⁻³¹.

How could dairy influence blood pressure?

Dairy foods are an important source of protein and minerals, and high-fat dairy contributes significantly to the intake of total and saturated fatty acids in the Netherlands⁴. There are several hypotheses on how dairy may lower blood pressure, but the underlying mechanisms remain to be established. A substantial body of evidence points to calcium as the main nutrient responsible for a beneficial effect of dairy on blood pressure, although the size of effect is relatively small. Meta-analyses of clinical trials documented modest reductions in systolic blood pressure of 1-2 mm Hg for calcium doses of 400 to 2000 mg/d^{24, 34-36}. Several mechanisms have been proposed on how calcium could be involved in blood pressure regulation^{37, 38}. Calcium-regulating hormones, e.g. parathyroid hormone could play a role. Calcium is also important for vascular smooth muscle contraction, and has been shown to induce natriuresis and prevent sodium-induced elevations in blood pressure.

Zemel hypothesized that dietary calcium plays a key role in blood pressure regulation in two ways; indirectly by influencing weight management and directly via suppression of 1,25-dihydroxyvitamin D, thereby reducing vascular smooth muscle intracellular

calcium, peripheral vascular resistance and blood pressure⁶. Findings from our observational analyses do not support an intermediary role of body weight, as discussed previously.

In the us, fortified milk is a main dietary source of vitamin D. A prospective population-based study by Wang et al. in 28,886 middle-aged us women showed that intakes of low-fat dairy products, calcium, and vitamin D were each inversely associated with risk of hypertension¹⁵. Since vitamin D enriched milk is not available in the Netherlands, we lack data to study the added value of vitamin D in dairy products in blood pressure control.

In both our observational studies (**chapters 2 and 3**), we observed an inverse relation of incident hypertension with low-fat dairy intake, but not with high-fat dairy. This differential association could not be explained by differences in calcium content, since that was more or less similar for e.g. semi-skimmed milk (123 mg Ca/100 g) and high-fat milk (116 mg Ca/100 g).

In countries where dairy intake is high, it may contribute to one fifth of the total daily intake of potassium and magnesium. These minerals have been shown to lower blood pressure in randomized controlled trials, with stronger and more consistent effects for potassium (2 to 6 mm Hg systolic) than for magnesium (1 mm Hg systolic)^{23, 39-43}.

Milk contains approximately 3.5% protein, of which ~80% casein and ~20% whey. Protein has been inversely associated with blood pressure in several observational studies⁴⁴⁻⁴⁶. In the Omniheart trial among 164 prehypertensive us subjects, partial substitution of carbohydrate with a mixture of animal and vegetable protein significantly lowered blood pressure⁴⁷. There is some evidence that high protein intake may influence body weight and glucose control⁴⁸⁻⁵⁰, which could partly mediate an antihypertensive effect. Dietary protein has also been shown to increase renal sodium excretion⁴⁴. Little is known about the specific effect of protein from dairy on blood pressure, but an effect on renal function, possibly by interaction with accompanying micronutrients (e.g. calcium, phosphorus) in dairy, has been suggested.

A fair amount of research into the blood pressure lowering effect of protein has addressed the ACE-inhibiting properties of milk, fermented milk or its peptides^{25, 26}. Although ACE-inhibiting properties of fermented milk or its peptides have been shown in *in vitro* and animal models⁵¹, recent data in human suggest that the plasma concentration of ACE-inhibiting peptides are far below the effective concentration for plasma ACE-inhibition⁵². In our trial, we found no effect of the lactotripeptides IPP and VPP on blood pressure, ACE-activity and angiotensin II (**chapter 4**). More studies that

could not confirm a beneficial effect of these two lactotripeptides on blood pressure or ACE-inhibition have recently been published^{27, 28}. The blood pressure lowering potential of milk-derived peptides is currently subject to debate and ACE-inhibition *in vivo* by these peptides is getting into doubt.

Up until now, total fat, saturated fat and industrial *trans* fatty acids have not been clearly associated with blood pressure and have therefore not been prioritized as an important blood pressure determinant⁵³⁻⁵⁷. The role of fats from dairy sources in blood pressure regulation is still unclear. *Cis-9, trans-11* conjugated linoleic acid (CLA), a *trans* fatty acid which is unique for dairy products, has recently attracted much attention because of the observed favorable effects on health (e.g. improved insulin sensitivity and blood lipid profile) in animal studies³². In addition, CLA was found to lower blood pressure in several rat models⁵⁸⁻⁶⁰. This could not be confirmed, however, in human intervention studies²⁹⁻³¹ and **chapter 5**. On the basis of current evidence, we consider dairy fat not a major factor in blood pressure regulation. More research is needed, however, on specific types of fatty acids in dairy, including short- and medium-chain length fatty acids that are abundant in ruminant milk fat.

Dairy also contributes to sodium intake (i.e., ~15% in the Netherlands). Sodium is an established risk factor for hypertension⁵³ and could counteract a potentially beneficial effect of dairy. We observed no association of cheese consumption with risk of hypertension in the MORGEN study (**chapter 2**) and in the Rotterdam study (**chapter 3**). To the best of our knowledge, studies showing an increased risk of hypertension or cardiovascular diseases with consumption of cheese have not been published.

Public health implications

Nutritional aspects of dairy

Dairy products are considered as nutrient-dense foods which can contribute substantially to daily intakes of nutrients such as calcium (~70%), vitamin B2 (~50%), vitamin B12 (~35%), protein (~25%) and potassium and magnesium (~18%)⁴. The average intake of dairy products in Dutch adults is 2-3 servings per day (~350-400 mL)⁴, which is below the current recommendation of 450-650 mL for milk and milk products⁶¹. The Dutch Nutrition Center and the Dutch Health Council acknowledge the contribution of dairy products to a healthy diet, with special emphasis on the use of reduced fat or skimmed alternatives⁶¹. In the US a daily intake of 3 servings of (fat-free or low-fat) milk is recommended⁶², but over the past decades a trend towards consuming less milk and more soft drinks has occurred and typical intakes are lower than recommended⁶³. In the US as well as in the Netherlands current recommendations are primarily directed towards the prevention of osteoporosis by ensuring an adequate intake of calcium.

Diet and blood pressure: the role of dairy

Diet and lifestyle are important for maintaining a healthy blood pressure⁵³. In line with previous studies on dairy intake and blood pressure, we observed that intake of low-fat dairy products may reduce the risk of hypertension (**chapters 2 and 3**). However, it should be noted that evidence has mainly been derived from observational studies, and controlled intervention studies are needed to confirm these findings. Although the DASH trial demonstrated that a healthy diet which includes low-fat dairy products can substantially reduce blood pressure, it is likely that this should be attributed to several aspects of the DASH diet, rather than just one nutrient or food²².

Based on our observational findings, an intake of 1 serving of low-fat dairy products per day seems adequate with regard to blood pressure. We did not observe a 'dose-response' type of relationship for low-fat dairy. With regard to (very) high intakes of dairy or specific dairy foods, there was no evidence for a harmful effect on blood pressure in our studies. Even though high-fat dairy products were not associated with an increased risk of hypertension, we are of the opinion that low-fat dairy products are to be preferred in view of the total cardiovascular risk profile (e.g. blood lipids, weight management). Based on the findings presented in this thesis, and evidence from the scientific literature, there is at present no need to adapt the current recommendations for milk and milk products for the specific purpose of hypertension prevention.

In both our observational studies the risk of hypertension was reduced by ~20%, corresponding to a reduction in systolic blood pressure of 3 mm Hg in a general population with an average systolic blood pressure of 126 ± 19 mm Hg. Small reductions in blood pressure, if applied to the entire population, could have a huge public health impact. A population-wide reduction in systolic blood pressure of 3 mm Hg has been translated into a 12% reduction in stroke mortality and a 9% reduction in mortality from coronary heart disease⁶⁴.

The risk reduction for hypertension that we found for low-fat dairy is in line with several other observational studies. Evidence in this field, however, is not yet conclusive and more prospective cohort studies are needed, preferably with multiple measurements of diet and blood pressure over time. Also, it would be worthwhile to investigate the blood pressure effect of low-fat dairy in a well-designed and controlled feeding trial, thereby avoiding other dietary changes. If this appears to be successful, a randomized controlled trial may also be conducted in an international multicenter setting so that the importance of low-fat dairy for hypertension prevention can be studied in populations with different habitual dairy intakes, e.g. US, Asia, and Europe. In case a beneficial effect of low-fat dairy is confirmed in randomized controlled trials, further research into dairy components could be initiated.

What was already known

- A healthy diet can substantially lower blood pressure.
- Observational studies suggest an inverse association of dairy intake with blood pressure, but findings are not conclusive.
- Calcium is considered the main dairy nutrient that is responsible for blood pressure reduction, although it has only a small effect.
- Lactotripeptides (IPP and VPP) lowered blood pressure in Japanese and Finnish (mildly) hypertensive subjects.
- Little is known about the effect of conjugated linoleic acid (CLA) on blood pressure

What this thesis adds

- Low-fat dairy intake may reduce the risk of hypertension by 20% in younger and older Dutch adults.
- There is no evidence for an antihypertensive effect of IPP and VPP in Dutch subjects with high-normal blood pressure or untreated hypertension.
- High *cis*-9, *trans*-11 CLA intake is unlikely to affect blood pressure in normotensive Dutch subjects.
- This thesis indicates the need for more prospective cohort studies and randomized controlled trials on low-fat dairy and blood pressure

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Summary

Summary

Blood pressure is a strong, independent and modifiable risk factor for cardiovascular and renal diseases. The worldwide prevalence of hypertension is increasing rapidly, which calls for effective public health measures throughout the entire range of blood pressure. Diet and lifestyle play an important role in maintaining a healthy blood pressure. Whether intake of dairy products could play a role in reducing population blood pressure remains to be established. The research described in this thesis was initiated to further investigate the role of dairy foods in relation to blood pressure in the Dutch population. The aims were [1] to examine the association of (specific types of) dairy food intake with blood pressure level and incident hypertension in two Dutch population-based cohorts (20-65 y and over 55 y of age) and [2] to assess the effect of two dairy components on human blood pressure, i.e. lactotripeptides (IPP and VPP) and *cis-9, trans-11* conjugated linoleic acid (CLA).

Previous observational studies conducted in Europe and the US generally suggest an inverse association between dairy intake and blood pressure or hypertension, although findings are not conclusive. The Netherlands is a country where a wide variety of dairy products is consumed, which allows detailed examination of the association of total dairy and specific dairy foods with blood pressure over a broad range of intake. In **chapters 2** and **3**, the association of dairy intake with blood pressure and risk of developing hypertension was examined using data from two Dutch population-based cohort studies. The average total dairy intake of young and middle-aged adults from the MORGEN study (**chapter 2**) and older adults from the Rotterdam study (**chapter 3**) was 350-400 mL (~2.5 servings) per day, ranging from less than 1 serving in the lowest category to ~5 servings in the highest category of dairy intake. Blood pressure level was not consistently related with overall dairy intake or intake of specific dairy foods in 21,553 Dutch adults aged 20-65 y from the MORGEN study (**chapter 2**). The risk of developing hypertension was examined in 3454 of these participants with 5 y of follow-up (**chapter 2**) and in 2245 older Dutch adults from the Rotterdam study with 6 y of follow-up (**chapter 3**). In both studies, the risk of hypertension was reduced by ~20% in subjects who consumed more than 150 mL (~1 serving) of low-fat dairy per day. Other dairy foods, i.e. fermented dairy, high-fat dairy, milk and milk products, and cheese were not consistently associated with risk of hypertension (**chapters 2** and **3**).

With respect to the blood pressure lowering potential of specific dairy components, promising results for milk-derived bioactive peptides (IPP and VPP) have been reported for Japanese and Finnish subjects with elevated blood pressure. In **chapter 4**, the blood pressure effect of the lactotripeptides IPP and VPP was assessed in an 8-week randomized double-blind controlled trial in 135 middle-aged Dutch subjects with elevated blood

pressure. After a 2-week run-in period on placebo, subjects were randomly allocated to 4 groups who received a daily dose of 200 mL dairy drink with 14 mg lactotriptides obtained by concentrating fermented milk, enzymatic hydrolysis, or chemical synthesis, or placebo for 8 weeks, followed by a 2-week wash-out. Blood pressure was not affected by intervention with lactotriptides. The mean difference (95% confidence interval) in systolic blood pressure response between the treatment and control group was 2.8 mm Hg (-2.6, 8.2) for lactotriptides obtained by fermentation, -0.5 mm Hg (-6.0, 5.0) for lactotriptides obtained by enzymatic hydrolysis, and 1.6 mm Hg (-3.9, 6.9) for synthetic lactotriptides ($p = 0.46$). Treatment did also not affect diastolic blood pressure, home blood pressure, 24-h ambulatory blood pressure, plasma ACE-activity or plasma angiotensin II. Thus, our study did not support the hypothesis of an antihypertensive effect of these specific peptides.

Another dairy component that has recently attracted much attention is *cis-9, trans-11* conjugated linoleic acid (CLA). Favorable effects of CLA on health, including weight reduction, insulin sensitivity, blood lipid profile, and blood pressure, have been reported in animal studies. Little is known about the effect of CLA in humans. Therefore, the objective of **chapter 5** was to study the effect of *cis-9, trans-11* CLA on human blood pressure in a randomized cross-over trial. Sixty-one young normotensive Dutch subjects were sequentially fed each of 3 diets for 3 weeks, in a random order, for a total of 9 weeks. Diets were identical except for 7% of energy (18.9 g in a diet of 10 MJ/day), which was provided either by oleic acid, by industrial *trans* fatty acids, or by *cis-9, trans-11* CLA. High intakes of *cis-9, trans-11* CLA did not affect blood pressure. The effect of the CLA diet on systolic blood pressure was +0.11 mm Hg (95% CI: -1.27, 1.49) compared to the oleic acid diet. The effect of the industrial *trans* fatty acid diet on systolic blood pressure was +1.13 mm Hg (-0.25, 2.51) compared to oleic acid.

The main findings, methodological issues and interpretation of the findings of the studies described in this thesis are discussed and put into public health perspective in **chapter 6**. In line with previous studies on dairy intake and blood pressure, the observational studies described in this thesis support the hypothesis that low-fat dairy may reduce the risk of developing hypertension. However, evidence in this field is not yet conclusive and more prospective cohort studies are needed, preferably with multiple measurements of diet and blood pressure over time. Additionally, well-designed and controlled feeding trials are needed to confirm a blood pressure lowering effect of low-fat dairy, if present. In case a beneficial effect of low-fat dairy is confirmed in randomized controlled trials, further research may focus on specific dairy components. With regard to dairy components, we conclude that the lactotriptides IPP and VPP and *cis-9, trans-11* CLA are unlikely to play an important role in blood pressure regulation.

Current dietary guidelines in countries like the US and the Netherlands include 2-3 servings of low-fat or fat-free dairy per day, which is mainly based on the prevention of osteoporosis by ensuring an adequate intake of calcium. Based on the findings presented in this thesis, and evidence from the scientific literature, there is at present no need to adapt these recommendations for the purpose of hypertension prevention.





Summary in Dutch (samenvatting)

Samenvatting

Bloeddruk is een belangrijke risicofactor voor het krijgen van hart- en vaatziekten en nierziekten. Een optimale bloeddruk is een systolische druk lager dan 120 mm Hg en een diastolische druk lager dan 80 mm Hg. Van hypertensie wordt gesproken wanneer de bloeddruk groter of gelijk is aan 140/90 mm Hg of wanneer antihypertensieve medicatie gebruikt worden. Het risico op hart- en vaatziekten en nierziekten neemt toe bij elke bloeddrukstijging, zowel bij hypertensieven als normotensieven. Dit begint al bij systolische bloeddrukwaarden van meer dan 115 mm Hg (wat geldt als een 'normale' bloeddruk). Een hoge bloeddruk is een omvangrijk gezondheidsprobleem in Nederland: een kwart van de bevolking is hypertensief en nog eens een kwart is prehypertensief. Een kleine verandering in de bloeddruk in deze populatie heeft grote gevolgen op de volksgezondheid: bij een daling van 5 mm Hg (systolisch) zou het aantal beroerten met een derde afnemen en het aantal infarcten met een vijfde. Maatregelen om de bloeddruk te verlagen zijn daarom zinvol en gewenst op iedere leeftijd en bij elke bloeddrukwaarde.

Voeding en leefstijl spelen een belangrijke rol bij het handhaven van een gezonde bloeddruk. Bij het streven naar een gezonde bloeddruk is het onder meer van belang om voldoende te bewegen, een gezond lichaamsgewicht te behouden, zuinig om te springen met zout, matig te zijn met alcohol en een gevarieerde en gezonde voeding te eten. De consumptie van zuivelproducten is een belangrijk kenmerk van het voedingspatroon in de westerse wereld, vooral in Nederland. Er zijn aanwijzingen dat zuivelproducten een rol kunnen spelen bij het handhaven van een gezonde bloeddruk. In een aantal grootschalige epidemiologische studies (maar niet in alle) wordt een lagere bloeddruk gevonden bij toenemende zuivelconsumptie. Of zuivelproducten daadwerkelijk een rol kunnen spelen bij het handhaven van een gezonde bloeddruk is echter nog niet overtuigend vastgesteld.

In dit proefschrift hebben we de mogelijke rol van zuivelproducten in relatie tot de bloeddruk onderzocht in de Nederlandse situatie. De doelstellingen waren [1] te onderzoeken of inname van (bepaalde typen) zuivel gerelateerd is aan de hoogte van de bloeddruk en het risico op het ontwikkelen van hypertensie en [2] te onderzoeken of specifieke zuivelbestanddelen, te weten melkpeptiden (IPP en VPP) en het vetzuur *cis*-9, *trans*-11 geconjugeerd linolzuur (Eng.: Conjugated Linoleic Acid, CLA), de bloeddruk kunnen beïnvloeden.

In de **hoofdstukken 2 en 3** van dit proefschrift hebben we onderzocht of de zuivelinname in Nederland samenhangt met de hoogte van de bloeddruk en het optreden van hypertensie. Dit hebben we gedaan in twee grote bestaande steekproeven van de

Nederlandse bevolking, namelijk: het MORGEN project en het ERGO (Erasmus Rotterdam Gezondheid en Ouderen) onderzoek. In deze cohorten hebben we de gebruikelijke zuivelinname vergeleken met de hoogte van de bloeddruk en het ontwikkelen van hypertensie over een periode van 2 tot 6 jaar. De gemiddelde zuivelinname van deelnemers aan het MORGEN project (**hoofdstuk 2**) en ouderen uit het ERGO onderzoek (**hoofdstuk 3**) was 350-400 ml per dag wat overeen komt met ongeveer 2 tot 3 porties zuivel. Deze zuivelinname varieerde van minder dan 1 portie tot circa 5 porties per dag, maar dit verschil in inname was niet duidelijk gerelateerd aan de hoogte van de bloeddruk in 21.553 Nederlandse volwassenen in de leeftijd van 20 tot 65 jaar (MORGEN project, **hoofdstuk 2**). Vervolgens onderzochten we het risico op het ontwikkelen van hypertensie in 3.454 van deze deelnemers die gedurende 5 jaar gevolgd werden (**hoofdstuk 2**) en in 2.245 deelnemers van het ERGO onderzoek die 6 jaar gevolgd werden (**hoofdstuk 3**). In beide onderzoeken hadden personen die meer dan 150 ml (~1 portie) magere zuivelproducten per dag gebruikten ongeveer 20% minder risico op het krijgen van hypertensie. De inname van andere typen zuivelproducten, bijvoorbeeld gefermenteerde zuivelproducten, volle zuivel, melk en melkproducten, en kaas waren niet duidelijk geassocieerd met het risico op hypertensie (**hoofdstukken 2 en 3**).

Met betrekking tot bloeddrukeffecten van specifieke zuivelbestanddelen zijn recentelijk veelbelovende resultaten gerapporteerd voor uit melk afkomstige bioactieve peptiden (IPP en VPP). Diverse onderzoekers hebben een antihypertensieve werking van deze peptiden aangetoond in Japanse en Finse personen met een verhoogde bloeddruk. **Hoofdstuk 4** beschrijft de resultaten van een interventieonderzoek naar het effect van deze melkpeptiden op de bloeddruk bij Nederlandse volwassenen die een verhoogde bloeddruk hadden waarvoor ze geen medicatie ontvingen. Op basis van loting werden 135 deelnemers ingedeeld in vier groepen en kregen gedurende 8 weken dagelijks 200 ml zuiveldrank te drinken. Drie van de vier groepen kregen een zuiveldrank met specifieke melkpeptiden (IPP en VPP) die op verschillende manieren waren verkregen; namelijk: door het concentreren van gefermenteerde melk, door enzymatische hydrolyse en door chemische synthese. De controlegroep kreeg 200 ml zuiveldrank zonder extra melkpeptiden. Gedurende het onderzoek werden allerlei metingen verricht waaronder de bloeddruk. De deelnemers wisten tijdens het onderzoek niet in welke groep ze zaten en wat hun bloeddrukwaarden waren. Ook de onderzoekers wisten niet wie welke zuiveldrank kreeg. Na afloop van het onderzoek bleek dat interventie met de zuiveldrank met extra melkpeptiden geen invloed had op de bloeddruk. Het gemiddelde effect (95% betrouwbaarheidsinterval) op de systolische bloeddruk was +2,8 mm Hg (-6,0; 5,0) voor melkpeptiden verkregen door fermentatie, -0,5 mm Hg (-3,9; 6,9) voor peptiden verkregen door enzymatische hydrolyse en +1,6 mm Hg (-3,9; 6,9) voor synthetische peptiden ($p = 0,46$). De interventie met melkpeptiden had ook geen effect op de diastolische bloeddruk, zelfgemeten bloeddruk (thuis, met een geblindeerde

bloeddrukmeter), 24-uur ambulante bloeddruk en op concentraties van hormonen van het bloeddrukregulerende renine-angiotensinesysteem. Hieruit concluderen we dat ons onderzoek eerdere bevindingen van een bloeddrukverlagend effect van deze specifieke peptiden niet ondersteunt.

Een andere component uit zuivel dat momenteel in de belangstelling staat is het vetzuur *cis-9, trans-11* geconjugeerd linolzuur (CLA). In dierexperimenteel onderzoek zijn gunstige effecten van CLA gevonden, waaronder gewichtsvermindering, een verbeterde insulinegevoeligheid en een verlaagde bloeddruk. Het is niet duidelijk of CLA ook dergelijke effecten heeft bij mensen. Het doel van **hoofdstuk 5** was daarom om het effect van *cis-9, trans-11* CLA op de bloeddruk bij mensen te onderzoeken. Dit werd gedaan in een volledig gecontroleerde voedingsproef, d.w.z. 90% van de voeding werd verstrekt door de universiteit, de overige 10% konden de deelnemers zelf kiezen uit een lijst van voedingsmiddelen. Een groep van 61 jonge, gezonde Nederlandse volwassenen met een normale bloeddruk kreeg achtereenvolgens 3 verschillende testvoedingen aangeboden voor een periode van 3x3 weken, in willekeurige volgorde. De drie voedingen waren identiek, met uitzondering van 7% van de energie (18,9 g in een voeding van 10 MJ per dag) die werd verstrekt in de vorm van oliezuur, industriële transvetzuren, of *cis-9, trans-11* CLA. De deelnemers kwamen gedurende 9 weken elke maandag t/m vrijdag naar de universiteit om hun warme maaltijd te nuttigen. De rest van de voeding (inclusief aanwijzingen voor bereiding) kregen de deelnemers mee naar huis. Gedurende het onderzoek werden allerlei metingen verricht, waaronder de bloeddruk. Er was geen verschil in de hoogte van de bloeddruk na de diverse testvoedingen. Het effect van de voeding met CLA op de systolische bloeddruk was +0.11 mm Hg (95% betrouwbaarheidsinterval: -1,27; 1,49) in vergelijking met de voeding die was verrijkt met oliezuur. Het effect van de voeding met industriële transvetzuren op de systolische bloeddruk was +1.13 mm Hg (-0,25; 2,51). Een hoge inname van het vetzuur *cis-9, trans-11* CLA lijkt de bloeddruk dus niet te beïnvloeden, in ieder geval niet op korte termijn.

De belangrijkste bevindingen, methodologische aspecten en de interpretatie van de bevindingen van de studies beschreven in dit proefschrift worden besproken in **hoofdstuk 6**. Ook de mogelijke betekenis voor de volksgezondheid en suggesties voor toekomstig onderzoek komen in dit hoofdstuk aan bod. De resultaten van ons onderzoek bevestigen de hypothese dat het gebruik van magere zuivelproducten het risico op het ontwikkelen van hypertensie mogelijk kan verlagen. Deze bevindingen moeten echter bevestigd worden in andere prospectieve onderzoeken met meerdere metingen van de voeding en de bloeddruk in de tijd. Daarnaast is het wenselijk om goed gecontroleerde voedingsproeven uit te voeren waarin wordt nagegaan of magere zuivel daadwerkelijk de bloeddruk verlaagt. Als het gunstige effect van magere zuivel wordt bevestigd kan

er verder onderzoek worden gedaan naar de antihypertensieve werking van specifieke bestanddelen van zuivel. Met betrekking tot componenten uit zuivel concluderen we dat de melkpeptiden IPP en VPP en het vetzuur *cis*-9, *trans*-11 CLA waarschijnlijk geen belangrijke rol spelen in de regulering van de bloeddruk.

In Nederland wordt geadviseerd om 2 à 3 porties magere of vetvrije zuivelproducten per dag te nuttigen. Dit is voornamelijk gebaseerd is op de preventie van osteoporose door het waarborgen van een adequate inname van calcium. Op basis van de bevindingen van dit proefschrift en de wetenschappelijke literatuur, is er vooralsnog geen reden om deze aanbevelingen te wijzigen voor de preventie van hypertensie.



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Mariëlle



About the author

Curriculum Vitae

Mariëlle Francis Engberink was born on February 18th, 1977 in Laren, the Netherlands. After completing secondary school at 'Erfgooiers college' in Huizen, she started the Bachelor's program in Nutrition and Dietetics at 'Hogeschool van Amsterdam'. As part of that study, she performed an internship dietetic counseling at Academic Medical Center, Amsterdam and was involved in a clinical trial on leptin and obesity in humans at Maastricht University (Bachelor's thesis awarded by Novartis in 1999). She obtained her Bachelor's degree in 1999 and enrolled in the Master program Nutrition and Health at Wageningen University. She performed a Master's thesis entitled 'Reproducibility of the individual response of serum lipids to cafestol in coffee-oil: a human intervention study' and obtained her Master's degree with a major in Epidemiology in November 2001. From 2002-2004, she was appointed as a junior research scientist at the Division of Human Nutrition, Wageningen University, where she investigated the role of body iron status in the development of atherosclerosis in collaboration with Radboud University, Nijmegen and Sanquin Blood Bank, Nijmegen. Since June 2004, Mariëlle has been involved in research on dairy intake and blood pressure as a PhD candidate at Wageningen University. During her PhD project, she performed the studies described in this thesis which included a randomized controlled trial of dairy peptides and blood pressure (sponsored by Unilever R&D). She also performed epidemiological analyses on dairy intake and hypertension in several large prospective cohort studies, in collaboration with research groups at the National Institute for Public Health and the Environment (RIVM), Bilthoven and Erasmus MC, Rotterdam. Part of her PhD project was nominated for the 'Foppe ten Hoor' young investigator's award in 2007. Mariëlle joined the educational program of the graduate school VLAG, chaired the PhD committee of the Division of Human Nutrition and was secretary of the research committee of the Division of Human Nutrition. She joined several (international) conferences in the field of nutrition, epidemiology and cardiovascular diseases, received a young investigator's travel grant by the International Society of Hypertension (Japan, 2006), and organized the Masterclass 'Dietary Influences on Blood Pressure' (VLAG/NZO, 2006). In 2008, she was selected for the European Nutrition Leadership Program. Since January 2008, she has been appointed as a postdoctoral fellow by the Top Institute Food and Nutrition (TIFN), Wageningen, where she is involved in a multicenter project on dietary protein and blood pressure.

Publications

Original research papers

- **Engberink MF**, Geleijnse JM, Wanders AJ, Brouwer IA. *A very high intake of conjugated linoleic acid, a trans fat from milk and meat, does not affect blood pressure in normotensive human subjects*. Submitted for publication.
- **Engberink MF**, Povel CM, Durga J, Swinkels DW, de Kort WL, Schouten EG, Verhoef P, Geleijnse JM. *Hemochromatosis genotype and atherosclerosis: increased susceptibility to iron induced vascular damage in C282Y carriers?* Submitted for publication.
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- **Engberink MF**, Geleijnse JM, de Jong N, Smit HA, Kok FJ, Verschuren WM. *Dairy intake, blood pressure, and incident hypertension in a general Dutch population*. *J Nutr*. 2009;139:582-7.
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Reviews

- Buijsse B, **Engberink M**. *Leptine geen wondermiddel*. *Voeding Nu*. 2002;2:11-6 [in Dutch]

Abstracts

- **MF Engberink**, MAH Hendriksen, FJA van Rooij, A Hofman, JC Witteman, JM Geleijnse. *Dairy Intake and Incident Hypertension: The Rotterdam Study*. *Circulation* 2008; 117:235.
- **MF Engberink**, DECM Verschuren, FJ Kok, JM Geleijnse. *Effect of ACE-inhibiting milk peptides on human blood pressure – systematic review*. *Circulation* 2006;113:318.
- **MF Engberink**, EG Schouten, FJ Kok, IA Brouwer, JM Geleijnse. *The effect of Angiotensin-converting enzyme (ACE)-inhibiting peptides in untreated hypertensive subjects: A randomized controlled trial*. 21st Scientific meeting of the International Society of Hypertension, Fukuoka, Japan, 2006.
- **MF Engberink**, JM Geleijnse, J Durga, P Verhoef, DW Swinkels, WLAM de Kort, EG Schouten. *Cys282Tyr mutation of the hemochromatosis gene is related to body iron status, but not to carotid intima-media thickness*. *Circulation* 2004;109:18.
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Educational program

Discipline specific activities

Courses

- Nutritional and lifestyle epidemiology, VLAG, Wageningen (NL), 2003
- Functional Foods, ABS Graduate School, Helsinki (Finland), 2006
- Regression analysis, NIHES, Rotterdam (NL), 2006
- Dietary influences on blood pressure, VLAG/NZO, Wageningen (NL), 2006
- Principles of epidemiologic data analysis, NIHES, Lunteren (NL), 2007
- Survival analysis, NIHES, Rotterdam (NL), 2007
- Concepts and methods in epidemiology, HNE, Wageningen (NL), 2007

Meetings

- 5th International Congress on Coronary Artery Disease (ICCAD) – from prevention to intervention, Florence (Italy), 2003
- Annual meetings NWO Nutrition, Arnhem, Deurne (NL), 2005-2007
- Annual meetings of the Netherlands Epidemiology Society (WEON), Rotterdam (NL), 2003; Wageningen (NL), 2005; and Maastricht (NL), 2007
- Prospective registration of trials in the Netherlands, Dutch Cochrane Centre, Amsterdam (NL), 2005
- Dairy a healthy choice, NZO, Ede (NL), 2005
- Nutritional and health claims, VMT, Maarsen (NL), 2006
- 1st Nutrition & Health congress, Amsterdam (NL), 2006
- 21st Scientific Meeting of the International Society of Hypertension (ISH), Fukuoka (Japan), 2006
- Annual Conference on Cardiovascular Disease Epidemiology and Prevention, American Heart Association, San Francisco, California (USA), 2004; and Phoenix, Arizona (USA), 2006
- Dairy and blood pressure, NZO, Ede (NL), 2008

General courses

- Written English, CENTA, Wageningen (NL), 2003
- Scientific writing, CENTA, Wageningen (NL), 2003
- Organizing and supervising student projects, OWU, Wageningen (NL), 2006
- Introduction course didactics, OWU, Wageningen (NL), 2006-2007
- European Nutritional Leadership Program (ENLP), Luxembourg (L), 2008

Optional courses and activities

- Preparation PhD research proposal, Wageningen (NL), 2004
- PhD Tour UK and Scotland, Division of Human Nutrition, Wageningen (NL), 2005

- PhD Tour USA, Division of Human Nutrition, Wageningen (NL), 2007
- Literature study program, Division of Human Nutrition, Wageningen (NL), 2004-2007
- Research presentations, Division of Human Nutrition, Wageningen (NL), 2004-2008
- Epidemiology research meetings, Division of Human Nutrition, Wageningen (NL), 2005-2008

Colophon

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