Cheese intake and risk factors for cardiovascular diseases and the metabolic syndrome

With particular reference to Gamalost intake and its effect on blood pressure

Inntak av ost og risikofaktorer for hjerte- og karsykdommer og det metabolske syndrom

Med fokus på Gamalost-inntak og effekten på blodtrykk

Philosophiae Doctor (PhD) Thesis

Rita Nilsen McStay

Department of Chemistry, Biotechnology and Food Science

Faculty of Veterinary Medicine and Biosciences

Norwegian University of Life Sciences

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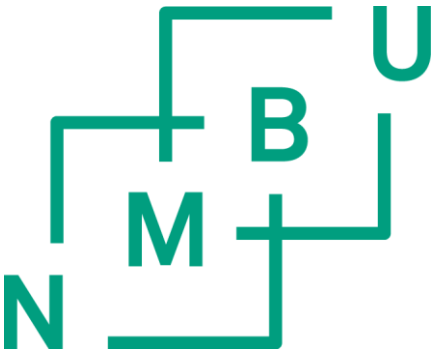

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Ås, February 2015

Rita Nilsen McStay

SUMMARY

The papers included in this thesis are part of a larger project titled “Healthy Cheese” (SUNN OST), with an overall main objective to establish a platform to develop innovative and healthy cheese varieties with reduced fat content, probiotic bacteria and bioactive components preferred by the consumers. More specifically, this PhD work aimed to investigate whether Gamalost, a cheese rich in bioactive peptides, could lower blood pressure in humans. Additionally, this work investigated whether a high intake of saturated fat from cheese had an effect on serum cholesterol levels.

Cheese, a concentrated milk product, is a good source of protein, fat and energy, as well as vitamins and minerals such as vitamin B12, calcium and phosphorus, but is also often high in both salt and saturated fat. As blood pressure is increasing worldwide and cardiovascular diseases currently contributing to approximately one third of all deaths, the dairy industry is expected to adapt and develop new products lower in salt and saturated fat. This manufacturing change will in turn influence both the texture and the flavour of cheeses that are marketed as “low-salt” or “low-fat”. Even though a high salt and saturated fat intake have been consistently linked to high blood pressure and high cholesterol, respectively, evidence has emerged in the last decade or so suggesting dairy products may have favourable effects on cardiovascular health.

Previous studies in the same project have shown that the traditional Norwegian cheese, Gamalost, is particularly rich in angiotensin-converting enzyme inhibiting peptides with a potential to reduce blood pressure. To assess whether the cheese could reduce blood pressure in humans it was decided to first to a cross-sectional trial on frequency of Gamalost intake and blood pressure (**paper I**). The study was carried out in Vik i Sogn, the town that produces Gamalost. Blood pressure was measured, along with all other parameters of the metabolic syndrome, and all participants filled in questionnaires about health, dietary habits and lifestyle. After adjusting for confounding factors, there was a small but significant association between lower systolic blood pressure and increasing frequency of Gamalost intake.

Since cross-sectional trials can only provide information on an association, it was decided to follow up with a larger randomized controlled trial to obtain better results on cause and effect. Both Gamalost and the Norwegian Gouda-type cheese, Norvegia, were included as interventions and measurements were taken at baseline and after 8 weeks of intervention. At the end of the trial, metabolic syndrome parameters and cholesterol levels were compared between the increased cheese intake groups and a control group of low cheese intake. A high cheese intake did not increase cholesterol or influence the parameters of the metabolic syndrome (**paper II**). Additional analyses stratified by the baseline presence or absence of metabolic syndrome showed slightly lower cholesterol levels in the Norvegia group compared to the control group. Blood pressure was measured additionally at four weeks, giving information on blood pressure development throughout the trial (**paper III**). At the end of the trial, blood pressure had decreased in all groups, but there was no significant difference between the cheese groups and control. At four weeks, when analysing the intervention groups based on baseline blood pressure category, diastolic blood pressure was lower in the Gamalost group compared with control for those participants who had borderline high blood pressure at baseline. In all intervention groups, systolic blood pressure continued to decrease slightly after four weeks, whereas diastolic blood pressure increased from four to eight weeks. It is possible that with a higher intake of Gamalost and 24-hour blood pressure measurements, we could have observed larger differences in blood pressure, however a larger intake would probably not have been feasible for most participants. However, it is also possible that bioactive peptides from cheese do not have the theoretical effect in humans when consumed as is.

SAMMENDRAG

Artiklene som er inkludert i denne avhandlingen er del av et større prosjekt med tittel SUNN OST, med det overordnete formålet å etablere en forskningsmessig basis for å utvikle innovative, sunne og markedsmessig interessante oster med redusert fettinnhold, probiotiske bakterier og bioaktive komponenter. Mer spesifikt, dette PhD-arbeidet hadde som mål å undersøke om Gamalost, en ost rik på bioatkive peptider, kan redusere blodtrykket hos mennesker. I tillegg ble det undersøkt om et høyt inntak av mettet fett fra ost hadde en effekt på serum kolesterolnivå.

Ost, et konsentrert melkeprodukt, er en god kilde til protein, fett og energi, i tillegg til vitaminer og mineraler som vitamin B12, kalsium og fosfor, men ost har også et høyt innhold av mettet fett og salt. Samtidig som befolkningens blodtrykk øker verden over og hjerte- og karsykdommer forårsaker omtrent en tredjedel av alle dødsfall, er det forventet at meieriindustrien tilpasser seg dette og utvikler nye produkter lavere på salt og mettet fett. Denne produksjonen av lett-oster og oster med mindre salt vil også forandre ostens konsistens og smak. Til tross for at et høyt inntak av salt og mettet fett konsistent har blitt assosiert med høyt blodtrykk og høyt kolesterol, respektivt, har det de siste årene blitt presentert forskning som tyder på at meieriprodukter kan ha gunstige effekter på hjerte- og karsykdommer.
Tidligere studier fra SUNN OST-prosjektet har vist at Gamalost er spesielt rik på angiotensin-konverterende enzym-hemmende bioaktive peptider, som har potensiale til å redusere blodtrykk, i forhold til andre oster. For å undersøke om osten kan ha denne effekten i mennesker ble det bestemt at man skulle gjennomføre en tverrsnittsstudie på frekvens av Gamalost-inntak og blodtrykk (**artikkel I**). Denne studien ble gjennomført i Vik i Sogn, bygda der Gamalost produseres. Blodtrykk ble målt, i tillegg til alle de andre parameterne i det metabolske syndrom, og alle deltakerne fylte ut spørreskjema som omhandlet helse, matvaner og livsstil. Det ble justert for konfunderende faktorer og resultatene viste at det var en liten, men signifikant, assosiasjon mellom lavere systolisk blodtrykk og økt frekvens av inntak av Gamalost.

Siden tverrsnittsstudier kun gir indikasjoner på assosiasjoner, ble det bestemt å gjøre en større randomisert, kontrollert studie for å få bedre resultater om årsak og effekt. Både Gamalost og

Norvegia ble inkludert som intervensjoner og målinger ble tatt ved inklusjon i studien og etter åtte uker med intervensjon. Ved avslutning av studien ble parameterne av det metabolske syndrom og kolesterolnivå sammenlignet mellom gruppene med økt osteinntak og kontrollgruppen med lavt osteinntak. Et høyt inntak av ost førte ikke til økning av kolesterol og hadde ingen effekt på metabolsk syndrom (**artikkel II**). Det ble i tillegg gjort analyser der deltakerne var stratifisert basert på om de hadde metabolsk syndrom ved studiestart og det ble funnet at deltakerne i Norvegia-gruppen hadde noe lavere kolesterolnivå enn kontrollgruppen ved studieslutt. Det ble gjort en tilleggsmåling av blodtrykk midtveis i studien (uke 4), som ga informasjon om hvordan blodtrykk utviklet seg gjennom studien (**artikkel III**). Ved studieslutt var blodtrykk redusert i hele studiepopulasjonen, men det var ingen signifikante forskjeller mellom ostegruppene og kontrollgruppen. Da gruppene ble analysert basert på blodtrykksnivå ved studiestart, var det etter fire uker et signifikant lavere diastolisk blodtrykk i Gamalost-gruppen sammenlignet med kontrollgruppen, for de deltakerne som hadde moderat høyt blodtrykk ved start. I alle intervensjonsgruppene fortsatte systolisk blodtrykk å synke gjennom studietiden, mens diastolisk blodtrykk økte fra fire til åtte uker. Det er mulig at vi med et høyere Gamalost-inntak og 24-timers blodtrykksmåling kunne observert større forandringer i blodtrykk, men et høyere inntak ville sannsynligvis ikke ha vært mulig for mange av deltakerne. Det er også en mulighet at bioaktive peptider fra ost ikke har den effekten hos mennesker som man antar utfra labforsøk.

LIST OF PAPERS INCLUDED

Paper 1

Rita Nilsen, Are Hugo Pripp, Arne Torbjørn Høstmark, Anna Haug and Siv Skeie (2014). Short communication: Is consumption of a cheese rich in angiotensin-converting enzyme-inhibiting peptides, such as the Norwegian cheese Gamalost, associated with reduced blood pressure? *Journal of Dairy Science*, 97(5):2662-8, doi: 10.3168/jds.2013-7479

Paper 2

Rita Nilsen, Are Hugo Pripp, Arne Torbjørn Høstmark, Anna Haug and Siv Skeie (2015). Effect of "Gamalost®", a cheese rich in angiotensin-converting enzyme (ACE)-inhibiting peptides, on blood pressure: results of a randomized trial. Submitted to: *Journal of Dairy Science*

Paper 3

Rita Nilsen, Arne Torbjørn Høstmark, Anna Haug and Siv Skeie (2015). Effect of a high intake of cheese on cholesterol and metabolic syndrome: results of a randomized trial. Submitted to: *Food and Nutrition Research*

ABBREVIATIONS

ACE	Angiotensin converting enzyme
BMI	Body mass index
BP	Blood pressure
CHD	Coronary heart disease
CHS	Cardiovascular health score
CI	Confidence interval
CLA	Conjugated linoleic acid
CVD	Cardiovascular disease
DASH	Dietary approaches to stop hypertension
GI	Gastrointestinal
HDL	High-density lipoprotein
IPP	Isoleucine proline proline
LDL	Low-density lipoprotein
LTP	Lactotripeptides
MetS	Metabolic syndrome
RCT	Randomized controlled trial
REK	Regional Ethics Committee
RR	Relative risk
SD	Standard deviation
T2DM	Type 2 diabetes mellitus
VPP	Valine proline proline

1. THEORY

1.1 Gamalost

1.1.1 History

Gamalost, which literally translates to “old cheese”, is a traditional Norwegian cheese, but the origin of the name remains uncertain. The first detailed description of the production of Gamalost was published in 1774, where it was claimed the name derived from the ripening time [1]. However, some also suggest that the cheese was so named due to the distinctive “old” appearance of the cheese. In fact now, compared to many other cheeses, Gamalost is quite a young cheese, with a ripening time of only 10-20 days. In terms of how long the cheese has been around in Norway, it certainly is old. Some suggest even the Vikings made Gamalost, whereas the first written accounts of Gamalost are from the sixteenth century.

Gamalost was traditionally made by women at small “farms” (sæter) throughout the Norwegian mountains. The cheese making was labour intensive and heavy work for the women and girls at the farms. The cows had to be milked, the milk had to be skimmed and the equipment needed to be cleaned, before the cheese making could even begin. Gamalost was made by heating soured skimmed milk and separating the curd and the whey. After the cheese was pressed, it was time to start the ripening process. The cheese was left to mould and it was turned daily and wiped down so the mould would grow inwards in the cheese. Back then (pre-industrialisation), the cheese was ripened for three to 10 months, by a variety of microorganisms present on the different farms.

Towards the end of the 1800s, the Gamalost production was largely moved to the dairies. Around the same time the first scientific paper on Gamalost was also published, by Johan Oluf Olsen [2]. During the first part of the 1900s many people were working on setting standards for ensuring a consistently good quality of Gamalost cheeses. It was decided on certain factors that made Gamalost what it is today, which will be presented in the production section.

Today, the cheese is celebrated as a traditional Norwegian product that has a protected designation of origin (“beskyttet geografisk betegnelse, matmerk.no). The cheese not only has

dedicated followers around the country, there is even an annual festival celebrating the cheese.

1.1.2 Production

Today, Gamalost is produced on a commercial scale at the dairy TINE Meieriet Vik (Vik i Sogn, Sogn og Fjordane). The cheese making process is illustrated in **figure 1.1.1**. The dairy receives the milk, which is then skimmed. The skimmed milk is acidified for 24 hours at 20°C, using a lactic acid starter containing *Lactococcus* (*Lc*.) *lactis* subsp. *lactis* and *Lc*. *lactis* subsp. *cremoris* [3]. The soured milk is then heated to 60°C and the mass is centrifuged so the curd and the whey can separate [1]. Using an impact mill, the cheese curd is milled into grains and placed into moulds to be cooked in whey at 90 to 95°C for 1-2 hours [3]. After setting in room temperature, the cheeses are removed from the moulds and moved to shelves in the mould room. They are then sprayed with the mould, *Mucor mucedo,* before they are moved into the storage room (22°C). After two days, the furry cheeses (**image 1.1.1**) are wiped down so the mould starts growing inwards as the colour of the cheese starts to change to light brown (**image 1.1.2**). After approximately 10 days (**image 1.1.3**), the cheeses are packaged in foil for further ripening in the cold room. The cheese is ready for the consumers approximately two to three weeks after the cheese making commenced. The finished cheese has a yellow-brown colour, is cylindrical, weighs approximately 600 grams, and it is quite grainy and dry. The mould which gives the cheese its characteristic appearance, *Mucor mucedo*, is highly proteolytic, which causes a great release of peptides from the protein. These peptides may have certain health benefits, which will be discussed in subsequent chapters.

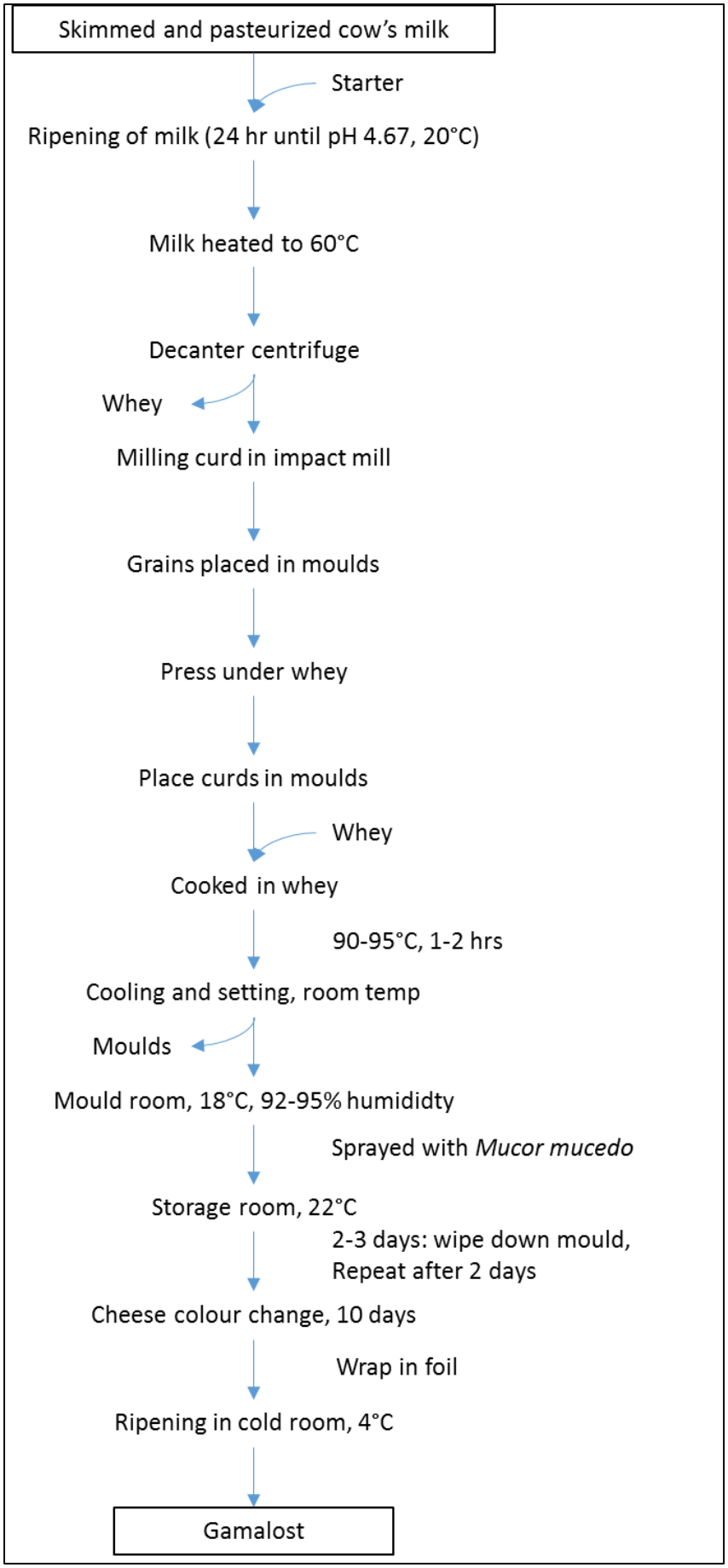

Figure 1.1.1 Flowchart of Gamalost making at TINE Meieriet Vik.

Image 1.1.1. Gamalost cheeses with the mould, Mucor mucedo, growing outwards.

Photo: Olav Håland, TINE.

Image 1.1.2. Gamalost cheeses after a few days of ripening. Photo: Olav Håland, TINE.

Image 1.1.3. Mature Gamalost, ready to be packaged. Photo: Olav Håland, TINE.

1.1.3 Composition

The nutritional value of Gamalost, according to TINE, is presented in **table 1.1.1** [4]. It can be seen that the cheese is made of 50% protein and contains very little carbohydrate and fat. Unlike other cheeses, Gamalost is also practically salt free.

Table 1.1.1. Nutritional value of Gamalost

Nutrient	Content per 100 g
Energy, kcal	213
Protein, g	50
Carbohydrates, g	1
Fat, g	1
Riboflavin, mg	1.5
Calcium, mg	160
Phosphorus, mg	600
Sodium, mg	24
Magnesium, mg	13
Potassium, mg	98
Zink, mg	3.3
Iodine, µg	80

1.1.4 Consumption

The consumption of Gamalost has decreased over the years. The dairy makes about 80 tons Gamalost annually, compared to 500 tons during the height of production. The typical Gamalost consumer is often described as “older and from Western Norway”. While this may be a stereotype, it does seem that Gamalost is more popular with the older population. In order to celebrate the cheese and attract new consumers, the dairy in Vik hosts the Gamalost festival every year, attracting both natives and tourists of all ages. Due to the dry texture and the bitter taste, Gamalost is traditionally eaten with a layer of butter both under and over the cheese. In fact, in a bit of exaggeration, Gamalost has been called “virtually inedible unless sliced very thinly” [5]. Many people also enjoy the cheese accompanied by some sour cream and berry jam, on bread or flat bread.

1.1.5 Gamalost as a health food

Talking to the people in Vik, one quickly discovers that the notion of Gamalost as a health food has been around for quite some time. One can hear tales of Gamalost being beneficial for numerous diseases, from impotence to wound healing to heart disease. Johan Ernst Gunnerus (1718-73), bishop of Trondheim, recommended Gamalost for diuretic purposes, as well as saying “there was nothing better for those who had eaten too many oysters” [5]. Whether or not some of these health claims have some truth to them will be further explored in the following sections and the papers.

1.2 Gouda-type cheeses

1.2.1 History

Unlike Gamalost, Gouda-type cheeses are very commonly consumed in Norway. Gouda is a Dutch cheese which was first introduced in some form in 1697 [6], but arrived in Norway some centuries later, in 1859 [7]. The first production of the cheese that would later become the most popular cheese in Norway, Norvegia (TINE SA), started at Nitedalen Ysteri before it spread to the rest of the country in the late 1800’s. It was a farmer named Sundt who first travelled to the Netherlands to learn about Gouda making [8]. However, Gouda got some competition from the Swiss cheese Emmental, and it was not until the late 1880’s that Gouda gained popularity again in Norway. At this time, the cheesemaking procedure was not

standardized, resulting in cheeses of varying and often poor quality. Dairy inspector Benterud was quoted after the first world war, on the lack of quality of Norwegian Gouda, saying "Norwegian cheese is either too fresh, or it is a soft, nauseating and sponge-like mass" [8].

The following years, work around standardization of Gouda cheese eventually led to Norway, in 1956, agreeing to make and sell Gouda at 45% fat in dry matter and the new name, Norvegia, was first proposed. The popularity of this Norwegian Gouda cheese increased in the first half of the twentieth century and in 1962 consumers were for the first time able to buy the square pre-packaged cheese with the new name Norvegia. In the 1990's Norvegia cheese was advertised as "Norway's most popular cheese", with a mild flavour that was suitable for the whole family. In 1996, Norvegia's biggest competitor was launched by Synnøve Finden AS, Synnøve gulost [9]. Today, we have a wide variety of Gouda-type cheeses, from low-fat to organic, from 6 weeks ripening to 15 months ripening.

1.2.2 Production

In Norway, there are several different producers of Gouda-type cheeses. The two main producers of in Norway are TINE SA and Synnøve Finden AS. For the purposes of this thesis, the TINE cheese Norvegia® will be used as the standard Gouda-type cheese. Gouda-type cheeses are made from pasteurized cow's milk and are characterized as having small eyes, being semi-hard in structure and salted in brine [10]. The first step in the cheesemaking process, as illustrated in **figure 1.2.1**, is the pasteurization and standardization of the fat content in the milk [10]. A mesophilic DL starter culture (*Lc. lactis* subsp. *lactis, Lc. lactis cremoris, Lc. lactis* subsp. *lactis* biovar *diacetylactis* and *Leuconostoc (Ln.) mesenteroides* subsp. *cremoris*) [6, 11] is added to acidify the milk, followed by addition of rennet to allow the milk to form a gel. After the gel has achieved the proper firmness, the coagulum is cut to a cube size of about 8-15 mm. The cubes are stirred for approximately 30 minutes in order to expel the whey from the cheese grains, a process known as syneresis. After the removal of the whey, hot water (42°C) is added, and the cheese curds are scalded at 38°C for approximately 40 minutes, to wash the curd of the remaining whey and to achieve the desired dry matter content. The second whey is drained before the cheese curds are pressed into moulds. After pressing the cheeses are placed in a brine (17-24% NaCl) in order to provide the cheese with

salt. The cheeses are typically ripened for at least 6 weeks or 2-3 months (depending on manufacturer), up to 24 months.

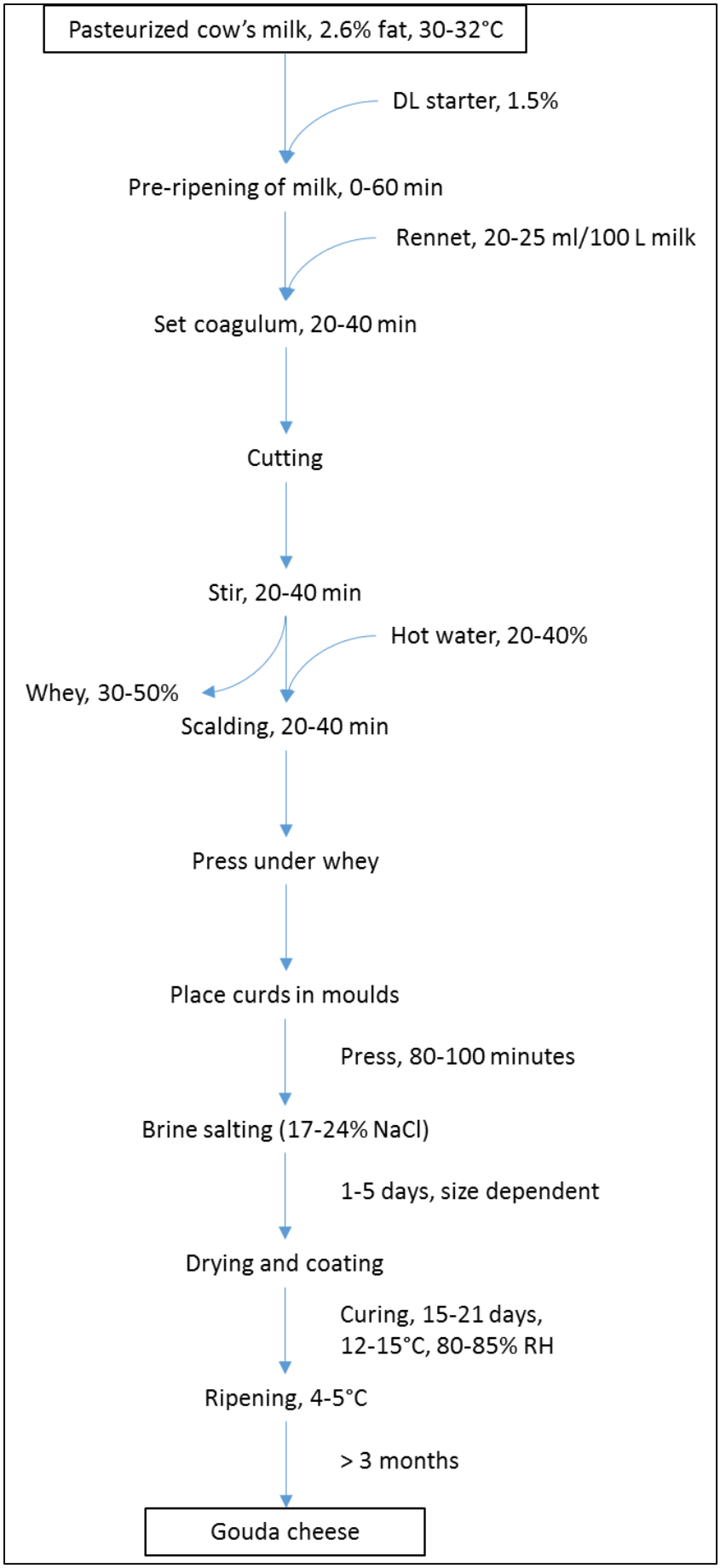

Figure 1.2.1. Flowchart of typical Norwegian Gouda-type cheese making. Adapted from Fox et al. (2000) and Skeie (2013) [6, 12]

1.2.3 Composition

The nutritional value of Norvegia, according to TINE, is presented in **table 1.2.1** [13]. The cheese contains equal amounts of fat and protein and is a good source of minerals such as calcium and phosphorus.

Table 1.2.1. Nutritional value of Norvegia

Nutrient	Content per 100 g
Energy, kcal	351
Protein, g	27
Carbohydrates, g	0
Fat, g	27
Saturated, g	17
Riboflavin, mg	0.31
Calcium, mg	820
Phosphorus, mg	600
Sodium, mg	402
Magnesium, mg	33
Potassium, mg	77
Zink, mg	4.6
Iodine, µg	31

1.2.4 Consumption

Numbers from 2013 show that the total turnover of Gouda-type cheeses ("gulost/hvitost") was 11.4 kg per person in Norway, with a total production of over 57 500 tonnes [14]. The cheeses are usually mild in flavour (depending on ripening time) and the texture and melting properties of the cheese makes it very versatile.

1.2.5 Gouda-type cheeses as health food

Gouda-type cheeses are an excellent source of energy, fat, protein, calcium, phosphorus and zinc and several vitamins. Still, with a saturated fat content of approximately 17%, they are typically not recommended in so-called "heart healthy" diets. The Dietary Approaches to Stop Hypertension recommend only a high intake of *low-fat* dairy [15], and the Dietary Guidelines for Americans recommends limiting saturated fat intake to 10 % of energy intake [16]. The evidence regarding the effect of cheese and dairy products on cardiovascular health will be presented in later sections.

1.3 Metabolic syndrome

1.3.1 Definition

The metabolic syndrome is a cluster of independent and interrelated risk factors for cardiovascular diseases (CVD) and type 2 diabetes mellitus (T2DM) [17]. While previously also described as "Syndrome X" [18] and "The Insulin Resistance Syndrome" [19], metabolic syndrome definitions were developed by the WHO (1999), The European Group for the Study of Insulin Resistance (1999), and the National Cholesterol Education Program – Third Adult Treatment Panel (ATP III) (2002) [20]. There is now a general agreement of what constitutes the metabolic syndrome and the cut points for each component have been clearly defined. For a person to receive the clinical diagnosis of the metabolic syndrome, he or she must meet at least three of the five criteria presented in **table 1.3.1**, according to the International Diabetes Federation; National heart, lung and blood institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International association for the study of obesity.

Table 1.3.1. Criteria for diagnosis of the metabolic syndrome. Adapted from Alberti et al, 2009 [17].

Component	Cut points
Elevated waist circumference	≥94 cm or ≥80 cm for men or women*
Elevated triglycerides	≥1.7 mmol/L
Reduced HDL-cholesterol	<1.0 mmol/L or <1.3 mmol/L for men or women
Elevated blood pressure	≥130 mmHg systolic and/or ≥85 mmHg diastolic
Elevated fasting blood glucose	≥5.6 mmol/L

*Europe. Population- and country- specific.

1.3.2 Prevalence

Due to the changing definitions through the years around the world, the prevalence of the metabolic syndrome in different populations will vary depending on the source used. The International Diabetes Federation estimates that as much as a quarter of the world's population have metabolic syndrome [21]. A publication based on European prospective cohort studies of non-diabetic men and women found a prevalence of 15% [22]. The prevalence of each individual component of the syndrome in the population varied between men and women in different age groups. Obesity was the least prevalent factor, only 13% and 18% for men and women, respectively. Hypertension was the most prevalent factor, with

almost half of the population having high blood pressure (BP). In the US, data from the National Health and Nutrition Examination Survey 1999-2004 show that the prevalence of metabolic syndrome in the normal weight population was 13.6%, whereas the prevalence was 39.2% for those who were classified with obesity class 3 [23].

1.3.3 Association with disease

Patients who meet the criteria for the metabolic syndrome have twice the risk of developing CVD over the next 5 to 10 years compared to persons who do not meet the criteria, and they are at five times the risk of developing T2DM [17]. Several factors contribute towards the development of metabolic syndrome, including both environmental and genetic factors [24]. Recently, the gut microbiota is also implicated as being an important feature of the pathogenesis of metabolic syndrome [25]. As shown in **figure 1.3.1**, these background factors first contribute to a positive energy balance, which leads to a growth of adipose tissue. Excess adipose tissue, or central obesity, contributes to high blood glucose, high BP, high triglycerides, low high-density lipoprotein (HDL)-cholesterol and insulin resistance, through a change in fatty acid metabolism and release of adipokines [20]. This change is also considered a state of chronic low-grade inflammation, which not only contributes to the development of the metabolic syndrome parameters but is also associated with CVD [26]. Animal models have found that our gut microbiota, which is influenced by our diet, is associated with obesity, insulin resistance and diabetes [27], but human trials have been inconsistent.

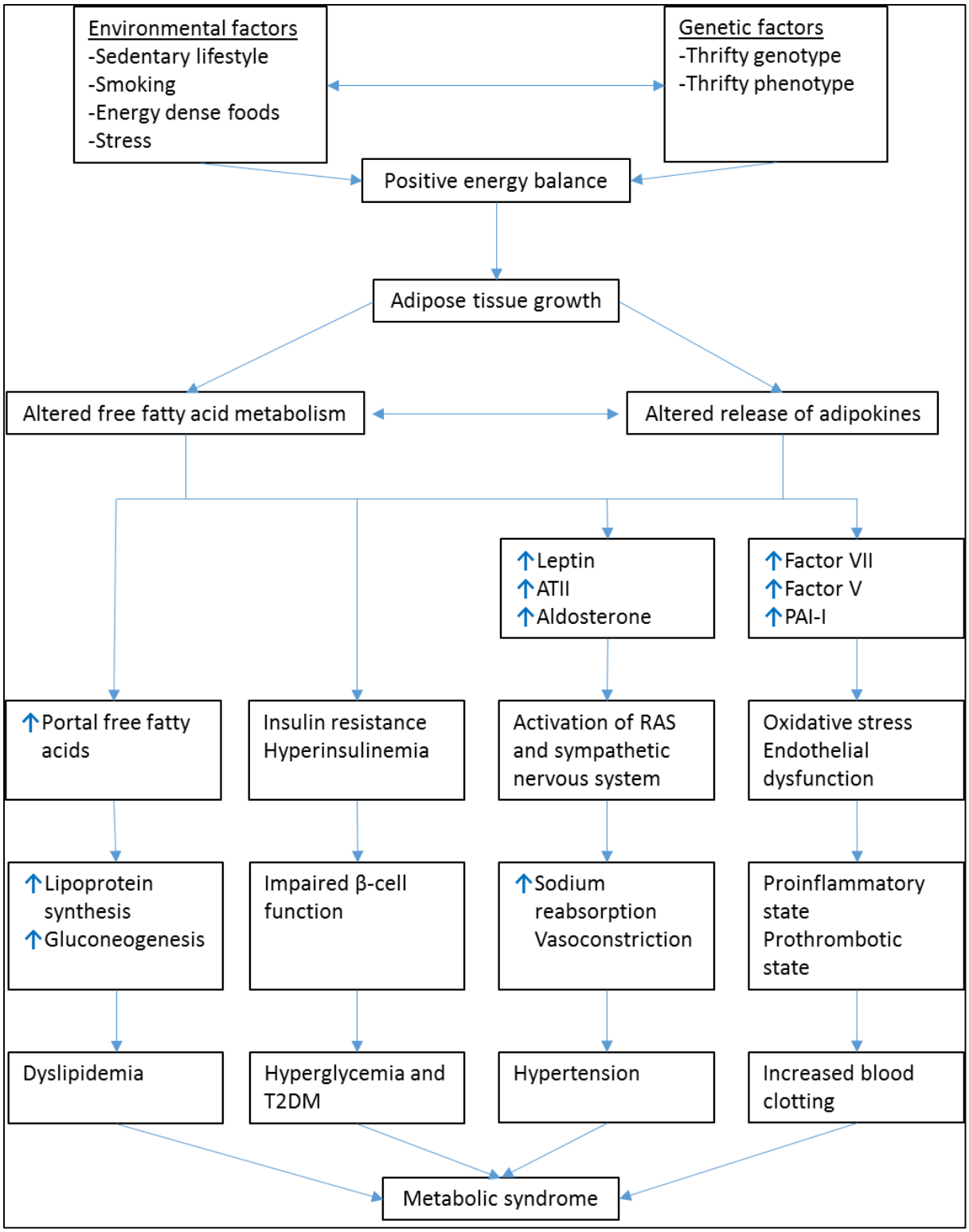

Figure 1.3.1. Independent and inter-related factors contributing to the development of metabolic syndrome. Adapted from Kaur, 2014 [24].

1.3.4 Treatment of metabolic syndrome

Prevention and treatment of the metabolic syndrome may be complicated due to the many independent factors contributing to the syndrome. The International Diabetes Federation states that the primary management strategy is healthy lifestyle promotion [20]. It is recommended to achieve a 7-10% weight loss over one year until a body mass index (BMI) <25 kg/m^2 is achieved, through a reduction of 500 to 1000 kcal/day [28]. At least 30 minutes, and preferably more than 60 minutes of aerobic activity daily is recommended [28], and to make dietary changes including reduced total and saturated fat intake, increased fibre and reduced salt if needed [20]. Pharmacological treatment of each of the metabolic syndrome parameters may be needed, depending on the severity of the problem. Even though a reduced intake of saturated fat is recommended, cheese and other dairy products have been shown to have positive impacts on the metabolic syndrome and its related factors [29], which will be further explored in the following sections and the papers.

1.4 Blood pressure

1.4.1 What is blood pressure?

The term blood pressure, or systemic arterial pressure, refers to the amount of pressure that circulating blood has upon the blood vessels [30]. Blood always flows from an area of higher pressure to an area of lower pressure, meaning the pressure varies throughout the circulation. The pressure is generated by the contraction of the heart, and the two factors affecting pressure is the cardiac output, i.e. the volume of the blood each ventricle pumps per minute, and total peripheral resistance, i.e. the sum of resistance by all the systemic blood vessels [30]. Historically, BP was measured by how high the pressure could force a column of mercury, thus naming the BP unit as millimetres of mercury (mmHg). As blood moves through the arteries the amount of pressure varies between maximum, also called systolic pressure, and minimum, called diastolic pressure (**figure 1.4.1**).

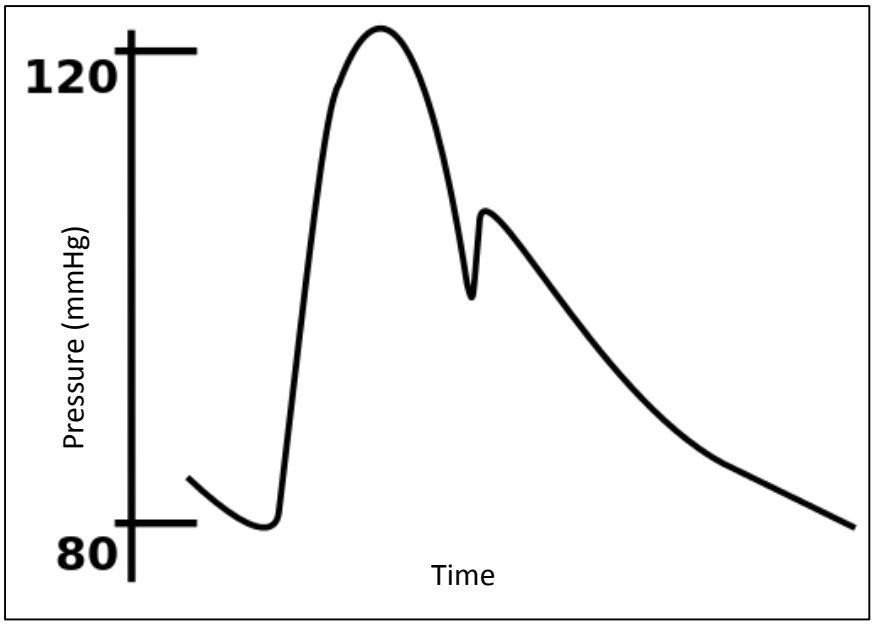

Figure 1.4.1. BP through one cardiac cycle. Adapted from Wikimedia Commons [31].

Several independent and interrelated physiologic factors influence arterial BP in the human body, as illustrated in **figure 1.4.2**. Some of these factors are, in turn, influenced by lifestyle factors such as physical activity and dietary choices. The main dietary factor which is often implicated in raised BP, sodium chloride, will not be greatly discussed in this work. In short, all adults are recommended to limit sodium intake below 2300 mg/day and people with raised BP or high risk groups should limit their intake to 1500 mg/day [16].

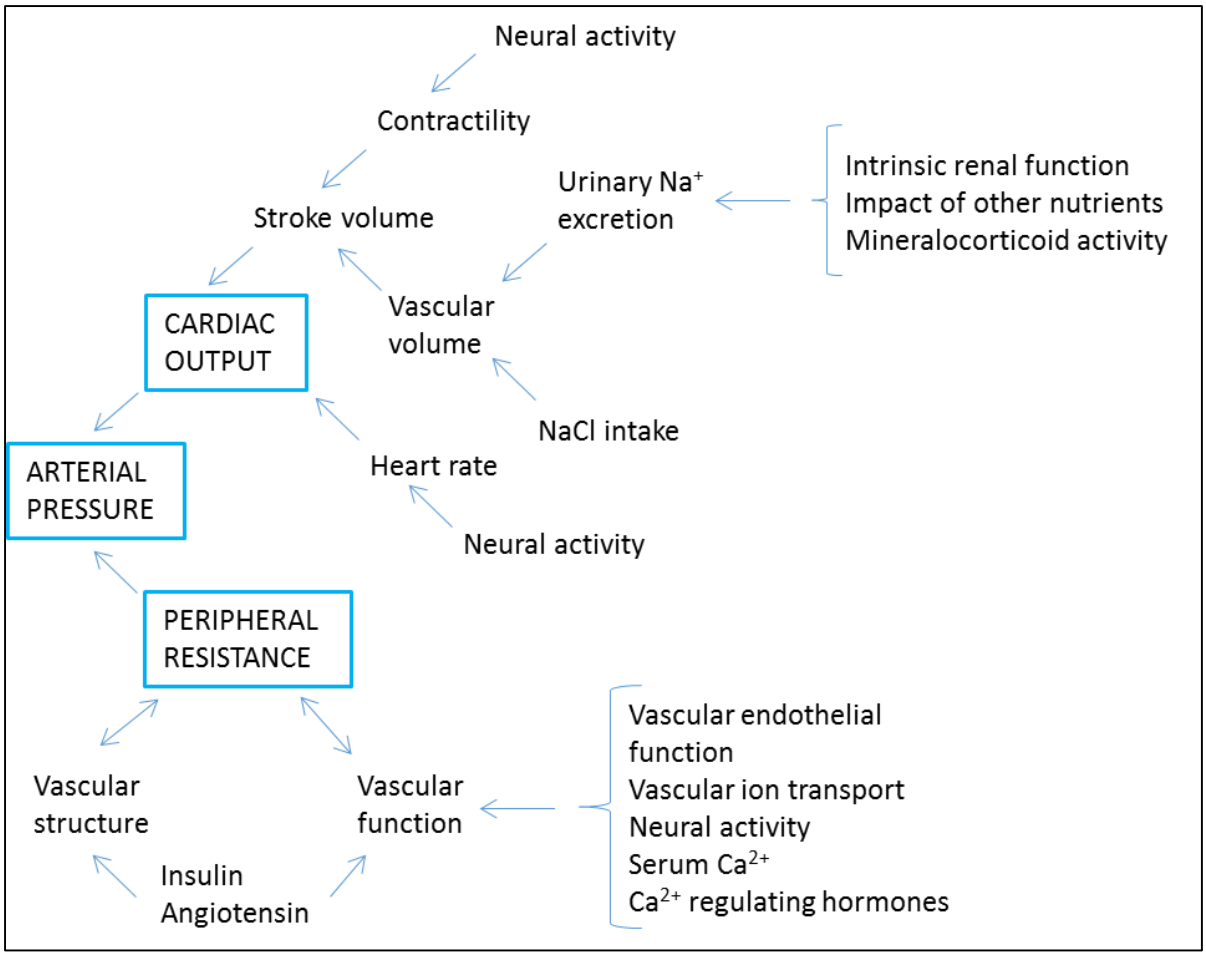

Figure 1.4.2. Factors influencing arterial BP. Adapted from Kotchen & Kotchen (2006) [32].

Blood pressure varies through the life cycle, with increasing prevalence of hypertension as age increases [33-35]. Systolic BP increases positively with age, whereas diastolic BP has more of an inverted U shape pattern, with the highest diastolic BP around 50-55 years [34].

1.4.2 Blood pressure in health and disease

Blood pressure was recently named the biggest contributor to the global burden of disease, up from fourth place in 1990 and surpassing childhood malnutrition in developing countries [36]. Some of the serious outcomes of raised BP are ischaemic heart disease, ischaemic stroke, aortic aneurysms and other cardiovascular diseases. It was estimated that the minimum risk was associated with a systolic BP of 110-115 mmHg (standard deviation (SD) 6 mmHg). In both males and females in 2010, it was estimated that 9 395 860 deaths were attributed to BP worldwide and 7 % of global disability adjusted life years. The same publication goes on to emphasise “the importance of implementing both population-wide and high-risk approaches

to reduction of blood pressure" [36]. This was also recognised in the most recent edition of the Dietary Guidelines for Americans, where it was estimated that about 50% of the adult US population would benefit from reducing sodium intake to less than 1500 mg/day, due to the high prevalence of high BP in many American populations and ethnic groups [16].

1.4.3 Hypertension

The clinical condition hypertension refers to a chronic increase of systemic arterial pressure [30], and is defined as BP exceeding 140/90 mmHg [37]. The most common type of hypertension is primary hypertension, meaning the cause is unknown, accounting for approximately 90% of hypertension cases. The prevalence of hypertension varies around the world, with one study showing a range from 28-31% in the United States to 44% in Western and Northern European countries [34, 38]. A recent study found that 40% of a Norwegian population (HUNT2 Nord-Trøndelag) had BP exceeding 140/90 mmHg [33]. The task force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC) recently published guidelines for the management of arterial hypertension which included BP categories [39]. These categories are intended to be used to classify people with varying degrees of BP so that they can receive the proper recommendations or interventions. Based on these categories, new and broader categories were used in the current work. The original and new categories are presented in **table 1.4.1**.

Table 1.4.1. Classification of BP levels (mmHg) from the 2013 ESH/ESC guidelines for the management of arterial hypertension and new categories as used in this work.

Category	Systolic		Diastolic	New category
Optimal	<120	and	<80	Optimal
Normal	120-129	and/or	80-84	Normal high
High normal	130-139	and/or	85-89	
Grade 1 hypertension	140-159	and/or	90-99	Hypertension
Grade 2 hypertension	160-179	and/or	100-109	
Grade 3 hypertension	≥180	and/or	≥110	
Isolated systolic hypertension	≥140	and	<90	

1.4.4 Consequences of high BP

Hypertension is the main risk factor for all types of stroke, the biggest cause of long term disability in the United States [38] and the estimated cost of high BP for 2011 was $46.4 billion [40]. The Framingham study, a large epidemiological prospective study, showed over 30 years

ago that elevated BP increased the risk of stroke [41]. It seems that chronic elevated BP, more so than acute hypertension, is what causes stroke. The mechanisms by which raised BP causes stroke depends on the nature of the stroke itself. High BP causes stress on the endothelium of the cerebral blood vessels, as well as impairing dilation of the vessels, contributing to increased risk of ischemic stroke [38], the most common type of stroke accounting for 87% of stroke cases in the US [40]. **Figure 1.4.3** shows a simplified overview of the mechanisms by which hypertension induces with stroke. These structural and functional changes to the cerebral blood vessels happen over time, as hypertension is left uncontrolled.

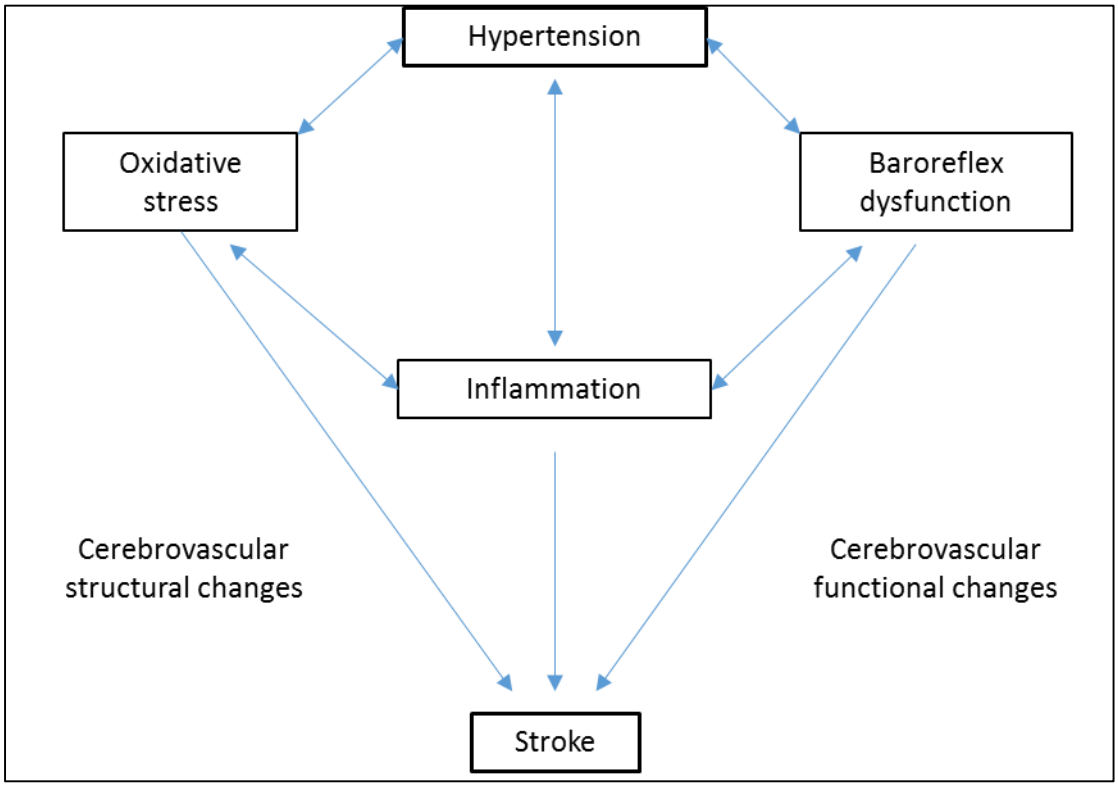

Figure 1.4.3. Mechanisms showing how hypertension induces stroke. Adapted from Yu et al (2011) [42].

Several clinical trials show very good reductions in the incidence of stroke when BP is properly managed. People who are pre-hypertensive/normal-high BP are also at risk for stroke and there is a positive association between BP and stroke risk even at normal levels of diastolic BP [43]. A meta-analysis from 2014 gathered results from 20 prospective cohort studies for a total sample population over one million, comparing relative risk for CVD of pre-hypertensive people with those with optimal BP [44]. In this meta-analysis, the prevalence of pre-hypertension ranged from 28.5 to 77.1% and the duration of follow-up was 5.1 to 36 years.

Being pre-hypertensive significantly increased the risk for CVD mortality (relative risk (RR) 1.28, 95% confidence interval (CI) 1.16-1.10), coronary heart disease (CHD) mortality (RR 1.12, 95% CI 1.02-1.23) and stroke mortality (RR 1.41, 95% CI 1.28-1.56). The risk was higher for those on the higher end of the pre-hypertension spectrum as well as those in the ethnic black sub group. Further calculations also indicated that 10.5% of deaths from CVD and 14.6% of deaths from stroke could be prevented by eliminating hypertension. These results emphasise the need to reduce BP in those with borderline high BP, not just those who are not diagnosed with hypertension. An older review of randomized controlled trials showed that patients who achieved a 10-12 mmHg reduction in systolic BP and 5-6 mmHg reduction in diastolic BP had a 38% lower incidence of stroke [45]. It was also found that the reduction in stroke could be observed just a few years after achieving a BP lowering [46]. Combined data from the large, well-known cohort of the Framingham Heart Study, and from the cross-sectional National Health and Examination Survey II, suggested that only a 2 mmHg reduction in diastolic BP would result in a 6% reduction in the risk of coronary heart disease and 15% reduction in risk of stroke [47]. In fact, stroke has in the past years gone down from third to fourth leading cause of death in the United States, attributed most likely to better control of hypertension [48].

1.4.5 The renin-angiotensin system

The renin-angiotensin system is a system which influences BP in two ways; by its effect on vasoconstriction, and on sodium and water retention [49]. An overview of the mechanisms of the renin-angiotensin system is illustrated in **figure 1.4.4**. The role of the enzyme renin is to split the decapeptide angiotensin I from the plasma protein angiotensinogen. Angiotensin I is an inactive peptide, but by the action of angiotensin-converting enzyme (ACE), a chloride dependent zinc-metallocarboxypeptidase, it is converted to the active angiotensin II [50]. ACE is also important in the kinin-kallikrein system, where it will cleave the C-terminal dipeptide from bradykinin, a peptide hormone formed by the enzymatic action of kallikreins on kininogen precursors [51]. Bradykinin is a strong vasodilator [52], thus BP increases by the action of ACE forming inactive fragments [53]. Two of the most important effects of angiotensin II are its roles as a vasoconstrictor and in stimulation of aldosterone, a steroid hormone which stimulates sodium reabsorption by the kidneys. ACE-inhibitors, illustrated by

green crosses in **figure 1.4.4**, are commonly prescribed as a pharmacological treatment of hypertension.

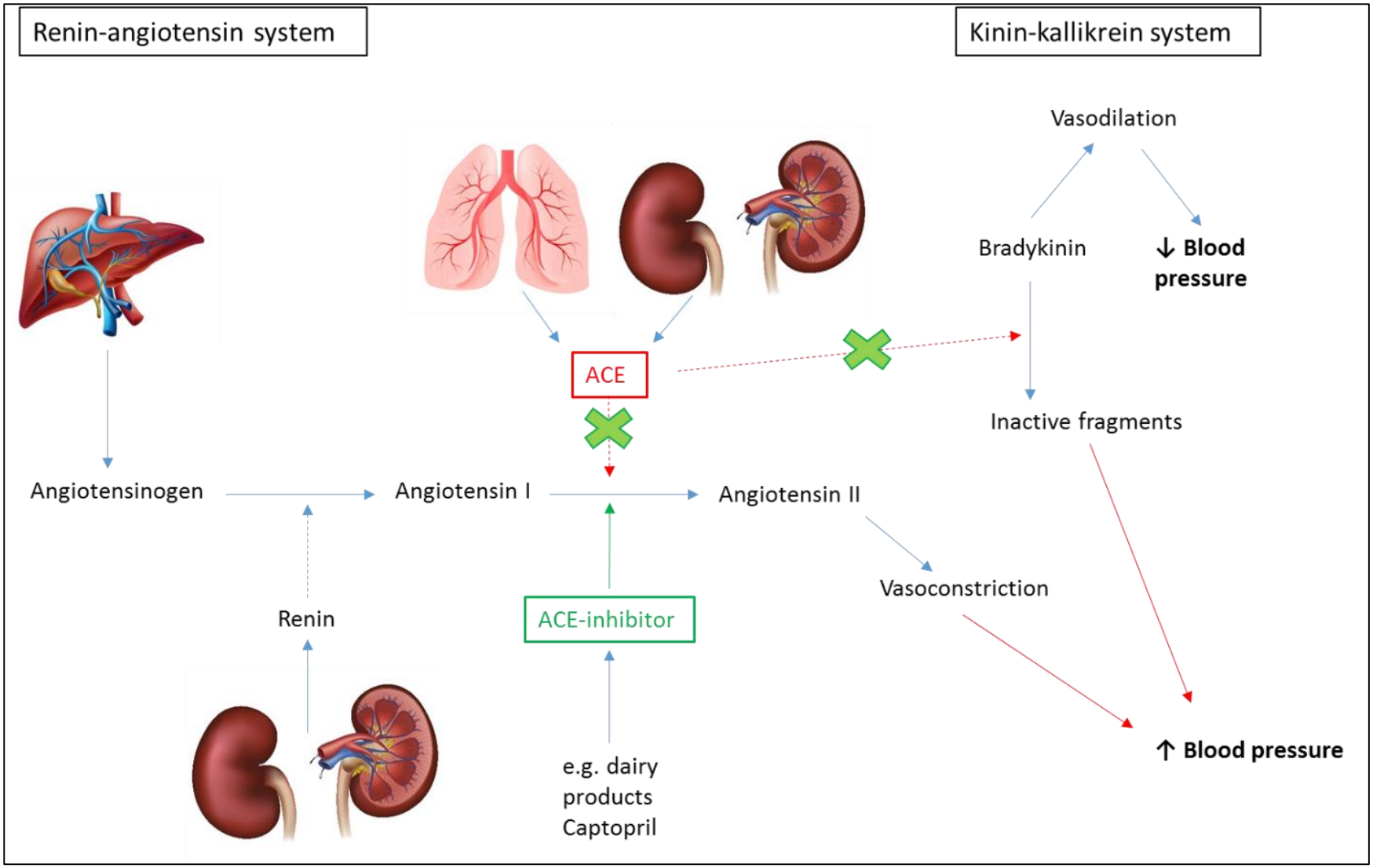

Figure 1.4.4. Renin-angiotensin system, kinin-kallikrein system and ACE-inhibitors: mechanism of action. Adapted from "Renin-angiotensin-aldosterone system" by A. Rad. Licensed under CC BY-SA 3.0 via Wikimedia Commons [54], pictures from Shutterstock.

1.4.6 Angiotensin-converting enzyme inhibitors

ACE-inhibitors (ATC number C09A, e.g. Captopril, Enalapril) are commonly prescribed pharmaceuticals used to reduce BP, and they are the first choice to use for uncomplicated hypertension in Norway[55]. Small doses are generally well accepted, whereas moderate to high doses can cause some side effects. Dry cough is the most common side effect, lethargy, nausea, and headache are other relatively harmless side effects [55]. More serious side effects include severe hypotension, however they depend on which ACE-inhibitor is used. Captopril, for example, lists renal failure as a side effect which may occur in rare cases (≥1/10 000 - <1/1000) [56].

A meta-analysis of randomized controlled trials on the effect of ACE-inhibitors on mortality in patients with diabetes mellitus showed that ACE-inhibitors reduced the risk of mortality from all causes by 13% (RR 0.87, 95% CI 0.78-0.98) [57]. Recently, much research has involved food derived ACE-inhibiting peptides. These peptides have been identified in many sources including mushrooms [58], turtle egg whites [59], and soy protein [60]. However, dairy products are often considered the best sources of these bioactive peptides, which is why dairy product intake is often investigated for their effect on BP. This will be further discussed in section 1.6.

1.5 Bioactive peptides

1.5.1 Definition

Bioactive components in milk are essential or non-essential substances that may have an effect on human health beyond the basic nutritional value of the milk [61, 62]. These substances include bioactive peptides, immunoglobulins, milk fat globule membrane proteins, oligosaccharides and some fatty acids (e.g. conjugated linoleic acid) [63], and can be found within the casein micelle, the lipid phase and the whey. These components are present in several dairy products, such as milk, cheese and yoghurt. Bioactive components can exhibit multiple functions on human health, and bioactive peptides from milk proteins have been the focus of much research. Bioactive peptides have been defined as specific protein fragments that have positive impacts on body functions or conditions and may influence human health [64]. The strategic zone of a milk protein is a peptide sequence that has one or more

functional roles [65], such as antihypertensive and antimicrobial. Examples of their bioactivities include opioid agonist or antagonist, antimicrobial, antithrombotic, immunomodulatory and ACE-inhibitory [66].

1.5.2 Release from proteins/ Production of peptides

The peptides are inactive within the protein, but can be activated in one of three ways: 1) through hydrolysis by digestive enzymes; 2) through hydrolysis by proteolytic microorganisms (starter and non-starter lactic acid bacteria used in cheese making); and 3) through the action of proteolytic enzymes derived from microorganisms or plants [67]. **Figure 1.5.1** illustrates how peptides can be released from their parent protein through the action of enzymes. This cleavage of peptides usually occurs during digestion of milk in the gastrointestinal tract, but also during fermentation and processing [65], as in the manufacture of cheese. Depending on the starter culture used in cheese making, different peptides are released from the protein. Starter cultures are proteolytic and consists of a mix of several cell wall-bound proteinases and intracellular peptidases which may cleave the proteins at different points in the amino acid sequence [68]. The bioactive peptides are generally small, usually from two to 20 amino acids long, and the physiological activity is dependent upon the sequence of amino acids [69]. As cheese ripens, different enzymes are active and the activity of the peptides changes. Therefore, the stage of cheese ripening greatly influences the potential of the cheese to have a function in the human body.

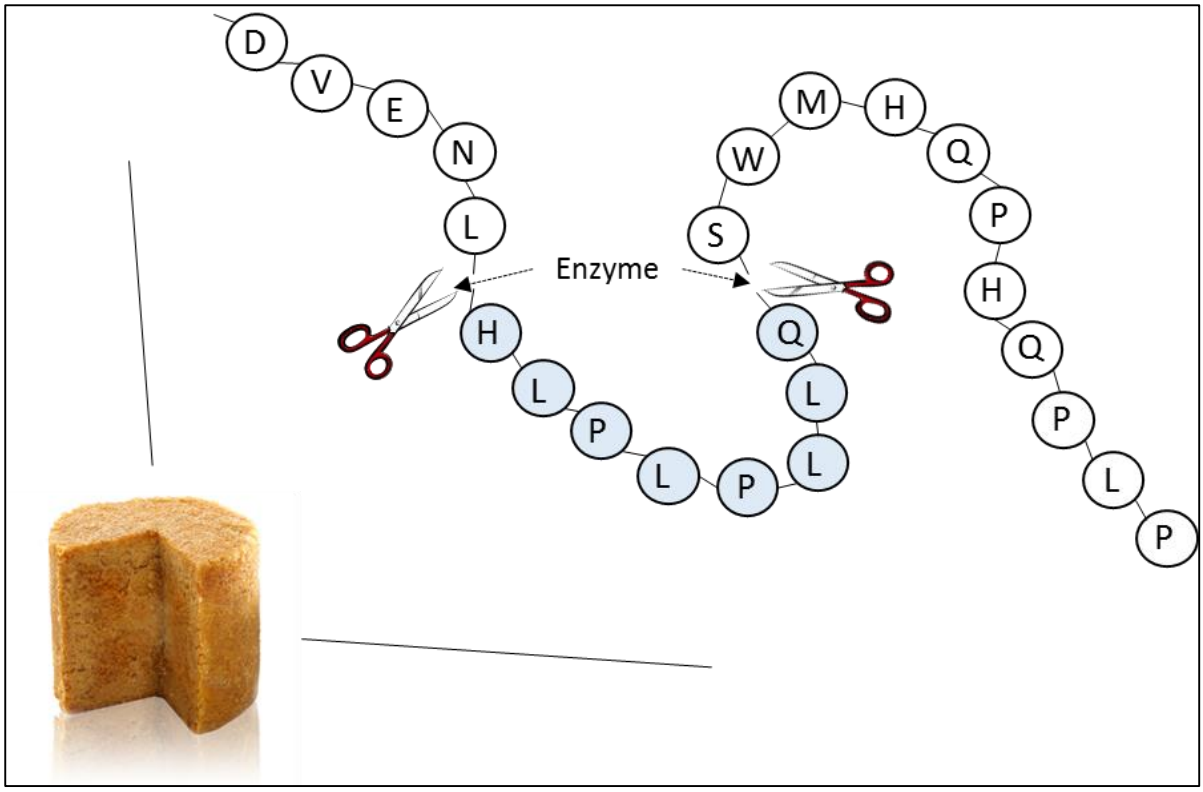

Figure 1.5.1. Illustration of release of ACE-inhibiting peptides from the parent protein (β-casein), by the action of enzymes.

1.5.3 Activities

Dairy products are considered the best sources of bioactive peptides, which may exhibit several physiologic activities in the human body, as illustrated in **figure 1.5.2**. Antimicrobial peptides, for example, have been identified in both whey proteins and caseins [65]. These antimicrobial peptides are generally amphiphilic with a positive charge, and their mechanism of action is to interact with bacterial membranes. Some dairy products have health claims based on specific bioactive peptides, including a caseinophosphopeptide to aid in mineral absorption, and a casein derived peptide for improved athletic performance [67]. The focus of this text will be on the antihypertensive activity and the potential role of ACE-inhibiting peptides on our cardiovascular health. These bioactive peptides have been identified in many cheeses, such as Cheddar [70], Asiago [71], Mexican Fresco [72], and Gamalost [3]. Gamalost, ripened by the highly proteolytic mould *Mucor mucedo,* is one of the cheeses with the highest ACE-inhibitory activity in terms of concentration of peptides from the cheese needed to inhibit 50% of ACE (IC_{50}) [73]. Making comparisons of the ACE-inhibitory potential of different cheeses is difficult, as the method of analysis often varies between papers. However, one trial

found that found that the ACE-inhibitory potential, expressed as mg captopril equivalents per kg cheese, was 0.61 for Gamalost, 0.12 for brie, and 0.08 for Norvegia aged 3 months [74].

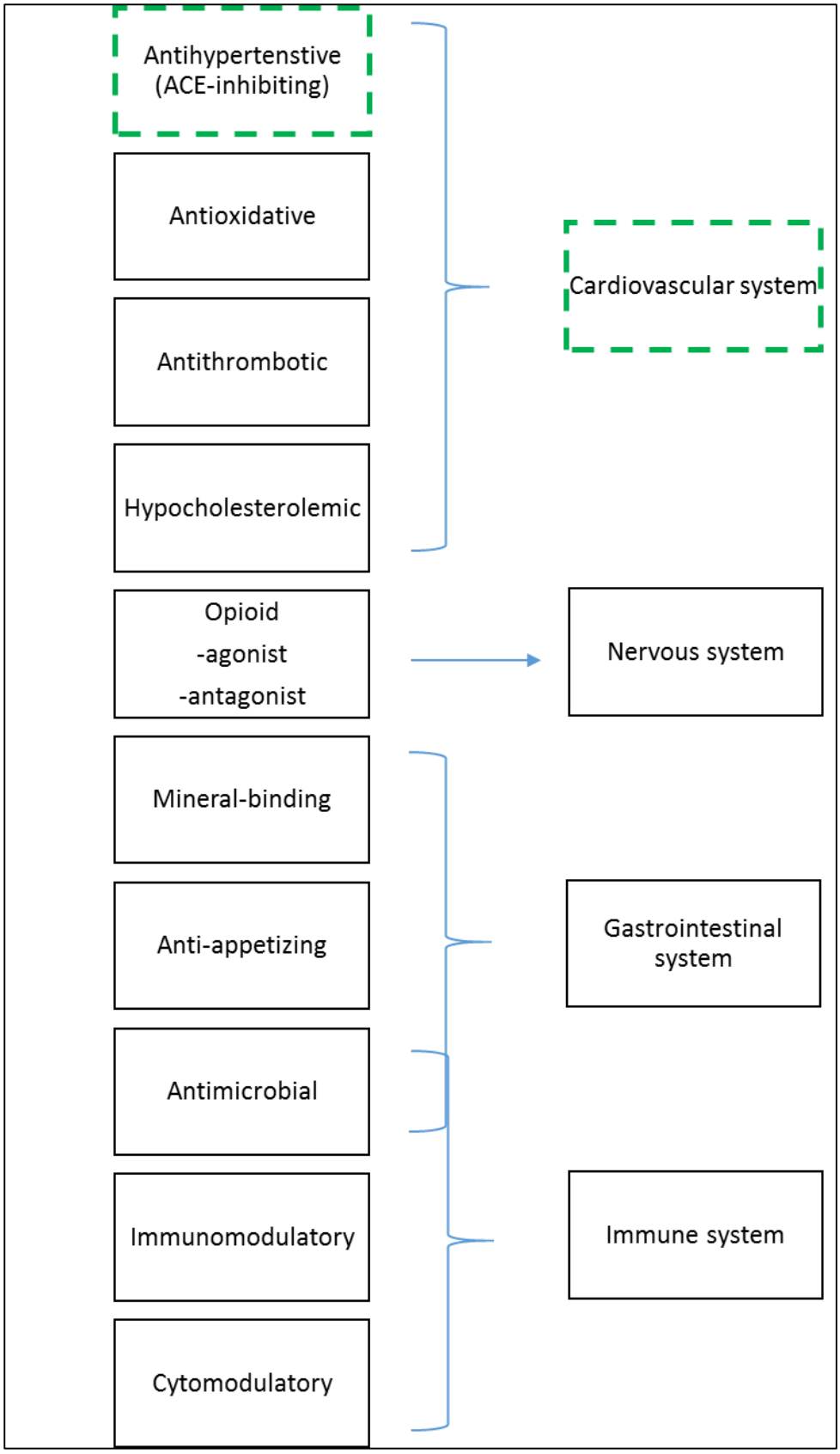

Figure 1.5.2. Functions of milk-derived bioactive peptides. Adapted from Korhonen & Pihlanto (2006) [67].

1.5.4 ACE-inhibiting peptides

ACE-inhibiting peptides, with the potential to decrease BP, are some of the most studied bioactive components in dairy products and other foods [75]. The strength of the bioactivity is usually expressed as IC_{50}, equivalent to the concentration of peptide required to inhibit 50% of ACE activity [76]. ACE-inhibiting peptides are usually small with low molecular masses and tripeptides appear to be more potent than other peptides [76]. Other characteristics of potent ACE-inhibiting peptides include: containing hydrophobic amino acids at the three C-terminal positions, an abundance of the amino acids tyrosine, phenylalanine, tryptophan, proline and lysine, and a high presence of isoleucine and valine [76]. The two best known ACE-inhibiting milk-derived bioactive peptides are isoleucine-proline-proline (IPP, IC_{50} = 5 µmol/L) and valine-proline-proline (VPP, IC_{50} = 9 µmol/L) [77]. Very little research has been done concerning the actual molecular mechanism of how these peptides may inhibit the enzyme. The molecular mechanism of a peanut protein derived ACE-inhibiting peptide has previously been established [78]. It was found that the peptide, with the sequence lysine-leucine-tyrosine-methionine-arginine-proline, docked in the active site of ACE, through hydrogen bonds, electrostatic bonds and Pi bonds. Furthermore, the peptide also formed carboxylic coordination bonds with the zinc atom present in ACE, thus inhibiting the enzyme. As far as the author is aware, no such work has been done regarding the molecular mechanism of dairy-derived ACE-inhibiting peptides.

A study investigating the ACE-inhibiting peptides of Manchego cheese looked at the effect of both the starter culture and the ripening time [79]. They made four cheeses: 1) raw milk without addition of bacterial starter, 2) pasteurized milk with a commercial mixed-strain starter (*Lc. lactis* and *Streptococcus thermophilus*), 3) pasteurized milk with *Lc. lactis* subsp. *lactis* (80%) and *Ln. mesenteroidetes* subsp. *dextranicum* (20%), and 4) pasteurized milk with *Lc. lactis* subsp. *lactis* (80%), *Ln. mesenteroidetes* subsp. *dextranicum* (10%) and *Lactobacillus plantarum* (10%). Whole cheese samples were taken from each batch at 15 days, 2, 4, 8 and 12 months ripening. They found that activity varied between the cheeses and the ripening time, showing the best ACE-inhibitory potential in raw milk cheese (1) aged 8 months. After 8 months the activity declined in all cheeses, but especially in the raw milk cheese.

Gamalost cheese, typically sold between 10-30 days of ripening, also has a varying ACE-inhibitory activity through ripening stage. Qureshi [3] investigated Gamalost at 0, 2, 5, 10, 20, 25 and 30 days of ripening and found that ACE-inhibition was significantly affected by ripening. The highest ACE-inhibition was measured at 10 days and it slowly decreased towards 30 days, but was still higher than at 0, 2 and 5 days.

Many studies have investigated the effect of ACE-inhibiting peptides on BP, from *in vitro*, to animal trials, to human observational trials to randomized controlled trials. Depending on the study design, product, follow-up and population, the results have been varying but there seems to be more trials showing positive results than negative results. Studies showing both negative and positive results are presented in the following section.

A Dutch trial of men and women with elevated systolic BP, randomized subjects to one of four groups: placebo (n = 32), or low-fat yoghurt drinks containing either fermented lactotripeptides (LTP) (n = 35), enzymatic LTP (n = 32) or synthetic LTP (n = 36) [80]. After 8 weeks of intervention there was no significant difference in BP between intervention groups and placebo. A smaller Japanese trial of hypertensive persons, randomized subjects to one of two groups: placebo (n = 13), or sour milk fermented by *Lactobacillus helveticus* and *Saccharomyces cerevisiae,* containing VPP and IPP (n = 17) [81]. After 8 weeks, there was no change in BP in the placebo group, whereas systolic BP was decreased by 14 mmHg and diastolic BP by 7 mmHg in the intervention group. A critical review of the effect of LTP on hypertension indicate that they are both safe and effective and can successfully be included as a lifestyle factor to prevent hypertension [77]. Few studies have investigated the BP-lowering effect of specific cheeses, but our results (presented in section 3.2 and 3.3) show that Gamalost cheese, with naturally occurring ACE-inhibiting peptides may be successful in lowering BP in a population. Results on the BP lowering effect of dairy products is further discussed in section 1.7.2.

1.6 Cholesterol

1.6.1 Recommendations

Cholesterol is a sterol which is essential in the structural integrity of the cell membranes, but too high serum levels have been associated with increased risk of heart disease. In Norway, it is recommended to keep total cholesterol levels below 5 mmol/L, however this is not always achieved. The average total cholesterol in 2000-2003 for 40 and 45 year olds was 5.7 mmol/L for men and 5.4 mmol/L for women, as measured in inhabitants of five counties in Norway [82]. This level has decreased since the 1980's and the level varies between different areas of Norway. In recent years, the focus has shifted from reducing total cholesterol levels, to reducing low-density lipoprotein (LDL)-cholesterol ("the bad") and increasing HDL-cholesterol ("the good").

1.6.2 LDL and HDL

Management of the level of serum LDL-cholesterol is the major goal of management of coronary heart disease [83]. Levels over 3.4 mmol/L is considered borderline high and above 4.9 mmol/L is very high [83]. Traditionally, the nutritional factors which have been associated with an increase in LDL-cholesterol are saturated and *trans* fatty acids, dietary cholesterol and excess body weight, whereas decreased LDL-cholesterol may be achieved by polyunsaturated fatty acids, viscous fibre, plant stanols and stenols, weight loss, and soy protein [84]. The principal recommendation for the dietary management of LDL-cholesterol levels by the American Heart Association is to replace saturated and *trans* fatty acids with dietary carbohydrate and/or unsaturated fatty acids [84]. However, in the past five or so years, many scientists and medical professionals have debated whether these are the optimal recommendations and if we should focus more on fat intake and eliminating sugar and other refined carbohydrates. This is also relevant in terms of cheese intake, where a high intake, and thus high intake of saturated fat, has not necessarily been proven to increase cholesterol levels (as discussed in section 1.7). As one of the diagnostic criteria for metabolic syndrome, having low HDL-cholesterol levels is associated with increased risk for CVDs. Having an HDL-cholesterol level above 1.6 mmol/L is considered the optimal level for minimising the risk of disease, however, there are no current European guidelines on a specific HDL-cholesterol level associated with the greatest decreased risk of CVD [85].

1.6.3 Cholesterol, calcium and cardiovascular disease

As will be discussed in section 1.7, dairy product intake has been associated with favourable changes in the risk of CVDs. One of the mechanisms suggested for this effect is through calcium intake. It has been found from rat studies that high amounts of dietary calcium can bind to bile and fatty acids, thus limiting their absorption and lowering cholesterol levels [86]. Most evidence on the effect of dietary or supplemental calcium on CVDs is from observational studies, and the results have been inconsistent. Some trials have shown an increase in CVD mortality from higher calcium intake, such as a Swedish cohort of women with a median follow-up of 19 years [87]. The highest intakes of calcium (≥1400 mg/day) was associated with higher cardiovascular mortality (hazard ratio 1.49, 95% CI 1.09-2.02) compared with intakes between 600 and 1000 mg/day. Other trials, such as results from NHANES III, show no association between dietary calcium intake and cardiovascular mortality [88]. Positive effects of calcium intake from dairy on CVD and cardiovascular mortality have been found in many trials, presented in section 1.7.

1.7 Dairy products and cardiovascular diseases

The "Norwegian recommendations for diet, nutrition and physical activity", published by The Norwegian Directorate of Health [89], aims to make recommendations ensuring optimal health for the Norwegian population based on scientific evidence. One of the main recommendations is regarding dairy intake, and is translated as follows: "Let low-fat dairy products be a part of the daily diet. Limit the use of dairy products with a high amount of saturated fat, such as whole milk, cream, full fat cheese and butter. Choose dairy products with little fat, salt and small amounts of added sugar." Thus, Norwegians are not recommended to consume cheese in excess. Two to three servings of low-fat dairy products per day is also recommended by the American Heart Association and the Dietary Guidelines for Americans [16, 90]. The Dietary Guidelines for Americans also recommend limiting intake of saturated fatty acids [16], of which cheese is the single biggest contributor in the US diet; 8.5% [91] or 16.5% [92] depending on the data used. However, cheese is also a significant source of essential nutrients, such as calcium, magnesium, phosphorus, vitamin B12 and vitamin A. Specifically, cheese contributes to 21% of total calcium intake, 11% of phosphorus

intake and 9% of vitamin A intake in the US [92] and 11% of calcium intake in the UK [93]. In recent years, evidence is emerging which suggest cheese may be healthier than the guidelines imply, which will be discussed in the following text.

1.7.1 Dairy and cardiovascular health

Several studies, mostly observational, have investigated the effects of dairy products intake on the prevalence and incidence of different cardiovascular diseases. A Swedish cohort of 33 636 women were followed for 11.6 years, and the authors investigated the effect of total and specific dairy food intake on the incidence of myocardial infarction [94]. Total dairy food consumption was significantly associated with a decreased risk of MI, an association which was also significant for cheese intake. They observed that women in the highest quintile of cheese intake, 6 servings/day, had a 26% lower risk of MI compared with those in the lowest quintile of only 0.7 servings/day. However, this association was attenuated after adjusting for calcium content, which suggests that calcium may be the main reason for this association. Another Swedish trial of the same cohort as well as a male cohort investigated the effect of dairy intake on stroke [95]. After a mean follow-up of 10.2 years they found no association for total or full-fat dairy or any single dairy product. Total low-fat dairy product intake was inversely associated with stroke risk (RR 0.88, 95% CI 0.80-0.97, *p* for trend 0.03). Since stroke risk is associated with BP, the authors suggested that the effect may be associated with a concomitant reduction in BP.

A cross-sectional trial of 1352 subjects from Luxembourg assessed the association with dairy product intake and cardiovascular health score (CHS) [96]. CHS, as defined by the American Heart Association, includes measures such as BMI, cholesterol, BP and fasting plasma glucose. It was found that total dairy and total high-fat dairy intake was positively associated with CHS, but this was not the case for total low-fat dairy. Total cheese intake was also positively associated with CHS ($p = 0.04$). These associations remained even after excluding participants who had modified their diet due to illness such as high BP and diabetes type 2.

Even though many trials show positive associations between dairy product intake and CVD, there are also instances of the opposite occurring. A Dutch cohort of 1956 subjects aged 50-75, free of CVD at baseline, was included in a study on dairy intake and mortality with a mean

follow-up of 12.4 years [97]. They found no association with total dairy intake and CVD mortality, however total high-fat dairy intake was significantly associated with a 32% higher risk of CVD mortality for each SD increase in intake (95% CI 7-61%). There were no significant association between cheese intake and CVD mortality, suggesting the association is caused by other high-fat dairy products. The authors suggested that the saturated fat content of high-fat dairy products was the cause of this association, but also acknowledged that there must be other unknown components or mechanisms behind this association. In Norway, dairy products are the biggest contributors to fat and saturated fat intake in the diet, accounting for approximately 1/3 of fat intake, with cheese alone accounting for 12% of fat intake in 2013 [98].

1.7.2 Dairy and elevated blood pressure

Low-fat dairy products are recommended as a part of the "Dietary Approaches to Stop Hypertension" (DASH diet) [99]. Dairy contains several nutrients which have been independently associated with BP reductions, such as protein, calcium, potassium and magnesium [100-103]. Furthermore, as mentioned previously, dairy products are also rich sources of ACE-inhibiting bioactive peptides which may reduce BP in humans.

1.7.2.1 Results from cohort studies

A large meta-analysis of five prospective cohort studies including 45 000 subjects in total, with over 11 000 cases of elevated BP, investigated the effect of total, low-fat, high-fat, and fluid dairy and cheese on BP [104]. They found that the highest intake category of total dairy (691 to 757 g/day) compared with the lowest resulted in 13% reduced risk of elevated BP (RR 0.87, 95% CI 0.81-0.94). When analysing low-fat and high-fat dairy products separately, the significant reduction remained for low-fat dairy only, whereas there was no change in risk for high-fat dairy products (RR 1.00, 95% CI 0.89-1.11) or cheese only (0.7-2.1 servings/day) (RR 1.00, 95% CI 0.89-1.12).

A dose-response meta-analysis of prospective cohort studies included some studies from the previously mentioned meta-analysis, as well as some additional studies, and aimed to investigate the effect of dairy consumption on incidence of hypertension [105]. Mean total

dairy intake ranged from 257 to 458 g/day and was linearly associated with incidence of hypertension (pooled RR 0.97, 95% CI 0.98-0.99, per 200 g/day dairy intake). Stratifying by BMI showed that the association was stronger in overweight individuals than normal weight persons. There were also some differences in type of dairy product: low-fat dairy was both linearly and inversely associated with incidence of hypertension, whereas there was no association for high-fat dairy. Approximately 51 000 individuals (including 15 000 hypertension cases) were included in the analysis of cheese intake, with mean cheese intake ranging from 10 to 43 g/day. There was no significant effect of cheese intake on the incidence of hypertension (RR 1.00, 95% CI 0.98-1.03, per 30 g/day cheese intake).

A population of older individuals with a hypertension prevalence of 80% at baseline found no effect of high-fat dairy on BP change over 12 months of follow-up. However, there was a significant effect of the highest (631.6 g/day) versus the lowest (3.1 g/day) quintile of low-fat dairy, with a -4.2 mmHg difference in systolic BP [106].

1.7.2.2 Results from intervention studies

There are few intervention trials investigating the effect of specific dairy foods such as cheese on BP or hypertension. A randomized, controlled 5-week crossover study of 62 subjects investigated the effect of low-fat dairy intake on BP in prehypertensive (84%) or stage 1 hypertensive (16%) subjects [107]. In the dairy period the subjects added to their standard diet one serving each of low-fat milk, low-fat yoghurt and low-fat cheese, while they in the non-dairy period they added an apple juice, a cereal bar and a pretzel. They assessed BP development from fasting to 3.5 hours postmeal of two servings of the intervention (dairy or non-dairy), after completion of the five week intervention period. There were no differences in systolic or diastolic BP postmeal between the test diets in the general study population or in subgroups of the study sample. The authors suggested that cheese intake may have attenuated the possible positive effect of yoghurt and milk on BP.

It has been shown that milk fermented with *Lactobacillus helveticus* is rich in the two bioactive lactotripeptides VPP and IPP [108]. A randomized, double-blind, placebo-controlled trial of tablets made from this powdered fermented milk investigated the effect on BP over 4 weeks [109]. There was no effect of the intervention in the subgroup with normal BP. In the group

with mild hypertension, systolic BP was reduced by 11.2 mmHg (95% CI 4.0-18.4, p = 0.003) compared to the placebo group at 4 weeks. This trial, using tablets instead of actual dairy products, largely eliminates blinding issues, as the placebo and test tablets were indistinguishable from each other. However, consuming a dairy product like a concentrated tablet may not be applicable to "real life", and it should be considered whether this moves away from an actual dairy product and into the supplement/pharmaceutical category.

1.7.3 Dairy and raised cholesterol

Full fat dairy products such as cheese are not recommended in "heart-healthy" diets due to the high content of saturated fat in these products. Saturated fat has for a long time been associated with increased serum cholesterol levels, and thus with increased risk of CVDs. However, studies have shown that consumption of dairy products, including full fat cheeses, are not consistently associated with raised cholesterol.

1.7.3.1 Results from cross-sectional and cohort studies

The American National Health and Nutrition and Survey III included 10 872 participants between the ages of 25 and 75 years who had data on cheese intake and blood lipids [110]. After adjusting for age, ethnicity, education, smoking, physical activity, menopausal status, servings of other foods high in saturated fat, and BMI, the authors investigated the effect of levels of cheese intake on blood lipids. They found no significant difference in total cholesterol across categories of cheese intake. Interestingly, there was a difference between men and women in the effect on LDL-cholesterol; with higher cheese intake, LDL was higher among men but lower among women (p for trend <0.05). In both men and women, HDL-cholesterol increased with increasing cheese intake (p for trend <0.05). The authors suggested calcium and conjugated linoleic acid as two of the factors which may have modulated the cholesterol raising effect of saturated fat.

A cross-sectional trial across three centres in France of 3078 participants aged 35 to 64 years investigated the effect of low-fat and high-fat dairy product intake on blood lipids [111]. They found that total and low-fat dairy were significantly negatively associated with LDL-cholesterol, but no effects were observed for high-fat dairy.

1.7.3.2 Results from intervention studies

A randomized cross-over trial of 14 young men investigated the effect of three weeks of consumption of three different dairy diets: high content of whole milk (1.5 L/ 10 MJ), butter (64 g/ 10 MJ), or cheese (205 g/ 10 MJ) [112]. Protein and lactose content were balanced in the three diets. After three weeks, butter intake resulted in 0.21 mmol/L higher LDL-cholesterol compared to cheese (p = 0.037). Total cholesterol was 0.20 mmol/L higher after butter intake compared to cheese, but this was only borderline significant (p = 0.054). Interestingly, there were no differences when comparing cheese with milk, or butter with milk. The authors suggested the high calcium content of the cheese diet could be contributing to the lower cholesterol, however this should also have been the case for the milk diet. Cheese being a fermented product was also suggested as a mechanism for the cholesterol lowering.

A very similar trial comparing butter with cheese also found similar results [113]. During the cheese intake period the 24 subjects consumed 150 g/ 8 MJ Jarlsberg cheese daily, or 52 g/ 8 MJ butter. Total cholesterol was lower after the cheese period than the butter period (-0.27 mmol/L, 95% CI -0.52, -0.015, p = 0.03). There was also a non-significant reduction in LDL-cholesterol after cheese compared with butter intake. Again, the authors suggested calcium or fermentation as the likely causes of this difference. The results from these two trials are also backed up from a similar trial showing a lowering of LDL-cholesterol when comparing cheese intake with butter intake [114]. This trial also showed that increasing cheese intake did not increase LDL-cholesterol compared with habitual diet pre enrolment in the trial, even though intake of saturated fat was increased.

1.8 Study design in human research

When conducting research with humans as the subjects, the study design generally falls within two categories: experimental or observational. The difference between the two categories is quite obvious: the former involves an experiment, whereas in the latter one is simply observing the subjects. The quality of study design, measured in terms of threat to internal validity [115], is often depicted in a pyramid with the highest quality study design at

the top (**figure 1.8.1**) [116]. However, even though RCTs have the least threat to internal validity, they are not always the most appropriate study design.

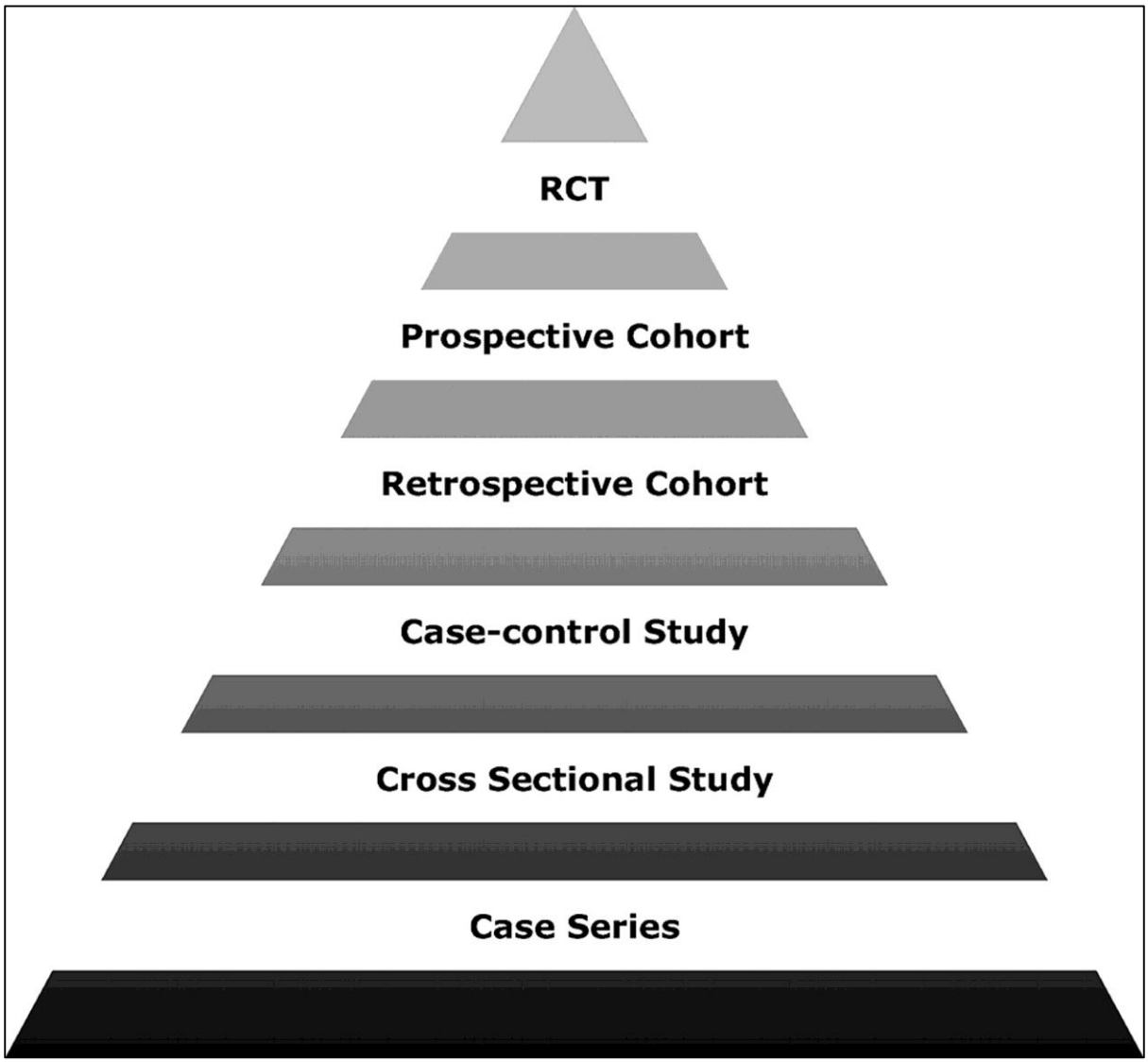

Figure 1.8.1. Hierarchy of evidence. From Circulation (2008) [116]

In nutrition research and evidence-based medicine it is important to evaluate whether evidence is good enough to provide a basis for recommendations on an intervention. For example, if a person is at risk for stroke, we must evaluate whether the evidence is good enough to provide a recommendation for increased dairy intake. The Oxford Centre for Evidence-based Medicine has produced a table on levels of evidence [117], aimed at evaluating the evidence behind a treatment. The strongest evidence for a therapy is produced by systematic reviews (or meta-analysis) of RCTs (level 1a), whereas individual RCTs with narrow confidence intervals are considered the second best option (level 1b).

1.8.1 Randomized controlled trials (study 2)

RCTs are considered the gold standard for testing whether an intervention is efficacious [116]. The subjects are randomly assigned to either a treatment group (e.g. cheese) or a control group, as illustrated in **figure 1.8.2**. The control group can receive a placebo which is undistinguishable from the treatment, or simply receive no treatment. Measurements of the outcome variable of interest (e.g. BP) are taken at inclusion of the trial and repeated at exclusion. The length of follow-up varies depending on the nature of the exposure and the outcome, with some trials only having a 24 hour duration while others may last up to a year or more.

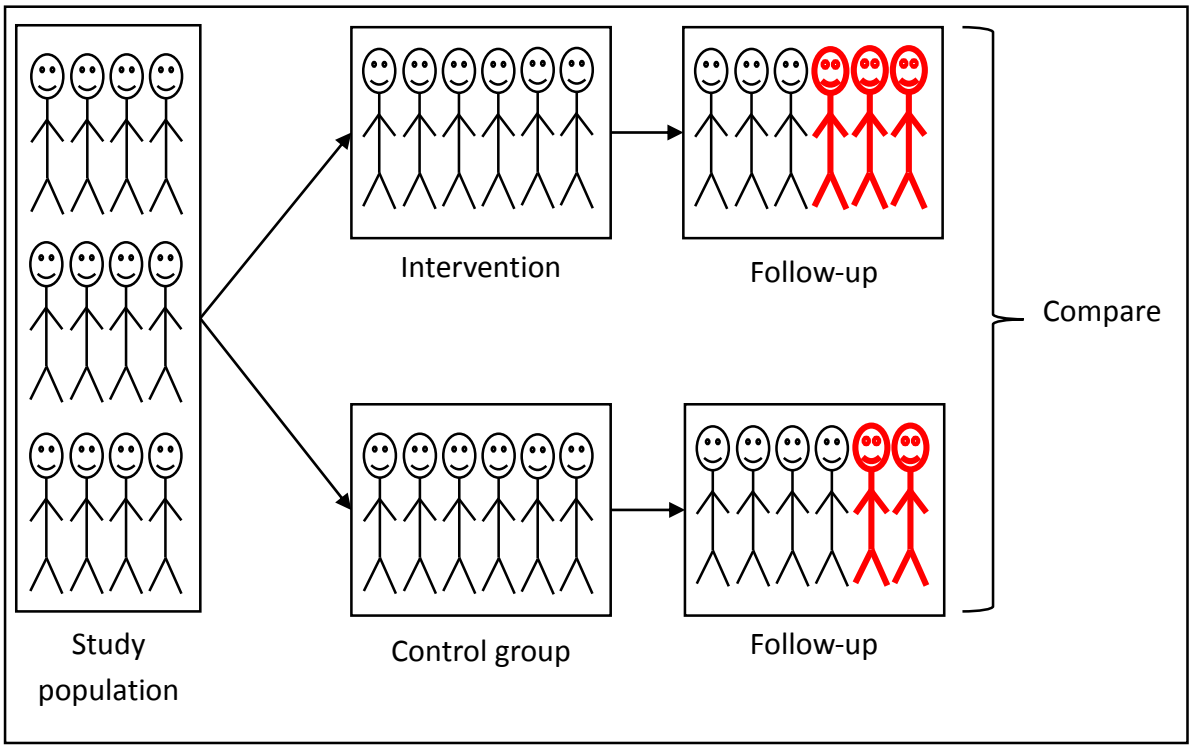

Figure 1.8.2. Basic principle of a randomized controlled trial.

An RCT must have a sample size large enough to ensure proper randomization with two (or more) equal groups. If the two groups are not equal at baseline, the risk of bias increases and the internal validity decreases. If the randomization has produced two equal groups confounding should not be a threat to the internal validity of the trial [116]. RCTs are prospective, meaning they are looking forward in time, which is a benefit because you can measure the development of an effect after the intervention has been assigned.

The best evidence is obtained from a trial which is double-blinded, meaning neither the participants nor the researchers know which group is receiving what treatment. However, it is not always possible to blind the participants because of the nature of the intervention. For example, in the trial on Gamalost and Gouda-type cheese (study 2) the participants were very aware of which group they were in. To overcome this issue, the trial may be single-blinded, meaning the researcher is unaware of which group is receiving the treatment or control. In the case of the Gamalost and Gouda-type cheese trial, the dataset was coded by an independent person so that data analysis was performed without knowing which group was which. The main strength of RCTs is that a properly conducted trial will produce evidence on cause and effect because the difference between the two groups are attributed to the intervention. However, RCTs are not always possible and may not be the best option for every research question. The main limitations include high cost, that they are time consuming and that only a few exposures and outcomes can be investigated [116]. Furthermore, depending on the intervention and the length of follow-up, participants may struggle with compliance and there may be loss to follow-up. Internal validity is usually very good, but if the study population is very narrow and specific, external validity may be low.

1.8.2 Cross-sectional trials (study 1)

Unlike RCTs, cross-sectional trials are simply an observation at one point in time, without any intervention or follow-up, and is often the first step of human trials. The exposure and the outcome are measured at the same time and the prevalence of the outcome, or the level of the outcome (e.g. BP), is compared between those with the exposure and those without, or between levels of exposure (e.g. frequency of Gamalost intake) [116]. Cross-sectional trials are often used to measure the prevalence of a disease in a given population, e.g. prevalence of hypertension in Oslo.

The benefits of cross-sectional trials are that they can include a large population at relatively small cost, that several exposures and outcomes can be measured simultaneously and that they are quite quick [116]. There is little work involved for the participants, as they usually only spend a day or two participating in the trial. Since these trials are just a point in time, we can only measure an association between the exposure and the outcome, but we cannot establish a cause and effect. The trials are subject to bias and confounding which necessitates

a statistical analysis that includes confounding factors in the model. For example, in the association between Gamalost intake and BP, confounders could include age, smoking and physical activity.

Details on the design of the cross-sectional trial and RCT included in this work are presented in the methods sections of the articles.

2. AIMS OF THE STUDY

The overall objective of this work was to obtain knowledge about the possible blood pressure lowering effect of cheese, and to investigate if cheese intake influences cholesterol levels.

Partial objectives:

- Study the effect of Gamalost, a cheese rich in ACE-inhibiting peptides, and Norvegia, a commonly consumed cheese with a lower ACE-inhibitory potential, on blood pressure in a human population (**papers I and II**).

- Study the effect of a high intake of a 28% fat Norwegian Gouda-type cheese on serum cholesterol levels in a human population, and if this effect was different from the effect of Gamalost, which is much lower in calcium and does not contain fat (**paper III**).

- Study the effect of cheese intake on variables of the metabolic syndrome (**paper III**).

3. MAIN RESULTS AND DISCUSSION

Previous studies have shown that cheese and other dairy products are often rich sources of ACE-inhibiting bioactive peptides, which have the potential to lower BP in humans. These peptides have been isolated and fed to hypertensive rats, showing promising results on BP reductions [73]. Drinks and pills/tablets containing large amounts of ACE-inhibiting peptides have also been fed to humans in RCTs, with results showing both a positive and neutral effect of the peptides [80, 81, 109]. We are not aware of any trials investigating specifically the effect of a cheese rich in ACE-inhibiting peptides, and its effect on blood pressure in humans. As such, the main aim of the thesis was to investigate the effect of Gamalost, a cheese rich in ACE-inhibiting peptides, and Norvegia, a regular Gouda-type cheese with a much smaller ACE-inhibiting potential, on human blood pressure (**papers I-II**). Additionally, since studies from the past 10 years show that cheese, although being high in saturated fat, may not raise cholesterol, we wanted to investigate the effect of the commonly consumed Norwegian cheese, Norvegia, on cholesterol and metabolic syndrome changes, and if this effect differed from Gamalost which is much lower in both fat and calcium content (**paper III**).

Trials involving human subjects are prone to limitations and challenges, either caused by the study design itself or by reporting errors from the subjects themselves. Many of the errors involving human subjects can be eliminated by conducting the study in a closed environment with complete control of all factors, however this is incredibly expensive and the results may not always be extrapolated to free-living populations. In the current work, it was decided to build upon previous laboratory experiments and conduct human trials involving two different study designs; first, an observational trial (**paper I**), and secondly and intervention trial (**papers II-III**).

3.1 Laboratory experiments on the ACE-inhibiting activity of Gamalost and Norvegia

Previously, Qureshi *et al.* (2012) [3] characterized the ACE-inhibitory activity of Gamalost during ripening from 0 to 30 days. It was found that ripening significantly affected the content of ACE-inhibiting peptides of the cheese, with the unripened cheese having the lowest ACE-inhibition. The ACE-inhibitory potential increased to 10-20 days of ripening, and was significantly lower after 25-30 days than after 10-20 days. The IC_{50} (amount of cheese needed

to obtain 50% ACE-inhibition) was lowest for Gamalost aged 10-20 days, and was almost half of the measured IC_{50} of 90 days ripened Norvegia.

Following the characterization of Gamalost, Qureshi *et al.* (2013) [118] performed an *in vitro* human gastrointestinal (GI) digestion of Gamalost and Norvegia and the effect on their ACE-inhibitory activity. Very few trials have been carried out using GI juices harvested from humans, as opposed to using mixes of commercial enzymes of non-human origin, but the results seem to be more similar to actual human digestion. As the previously mentioned trial, Gamalost at different stages of ripening were compared with Norvegia at 90 days of ripening. The ACE-inhibitory activity was highest after gastric digestion, but subsequent duodenal digestion did not affect the ACE-inhibitory activity of Gamalost any further. Interestingly, the IC_{50} of Norvegia decreased drastically after gastric and duodenal digestion, but the IC_{50} values were still higher than Gamalost at every step of digestion. Some of the peptides identified in Gamalost matched previously reported ACE-inhibiting peptides, and the two potent ACE-inhibitors, VPP and IPP, were present within the sequences of some of the identified peptides. It was decided that after the thorough investigations by Qureshi *et al*. (2012, 2013), it was time to test the hypothesis that cheeses rich in ACE-inhibiting peptides, like Gamalost, could reduce BP in humans.

3.2 Cross-sectional trial on Gamalost intake and blood pressure

The first trial we conducted was a cross-sectional trial on frequency of Gamalost intake and the effect on BP (**paper I**). Gamalost is not widely consumed amongst the Norwegian population, hence we decided to conduct the trial in Vik i Sogn, home of Gamalost production and with many Gamalost consumers. A cross-sectional trial was a reasonable first step, as they are often inexpensive, relatively quick, and they do not require much work or time commitment from the participants. Ethical approval was acquired from the Regional Committees for Medical and Health Research Ethics (REK), who evaluated the appropriateness of the study design. Details on the study design can be found in the paper itself (**paper I**).

Upon analysis of the collected data of 168 subjects, it was quickly discovered that, as expected, Gamalost intake was closely related to age, as illustrated in **figure 3.1**.

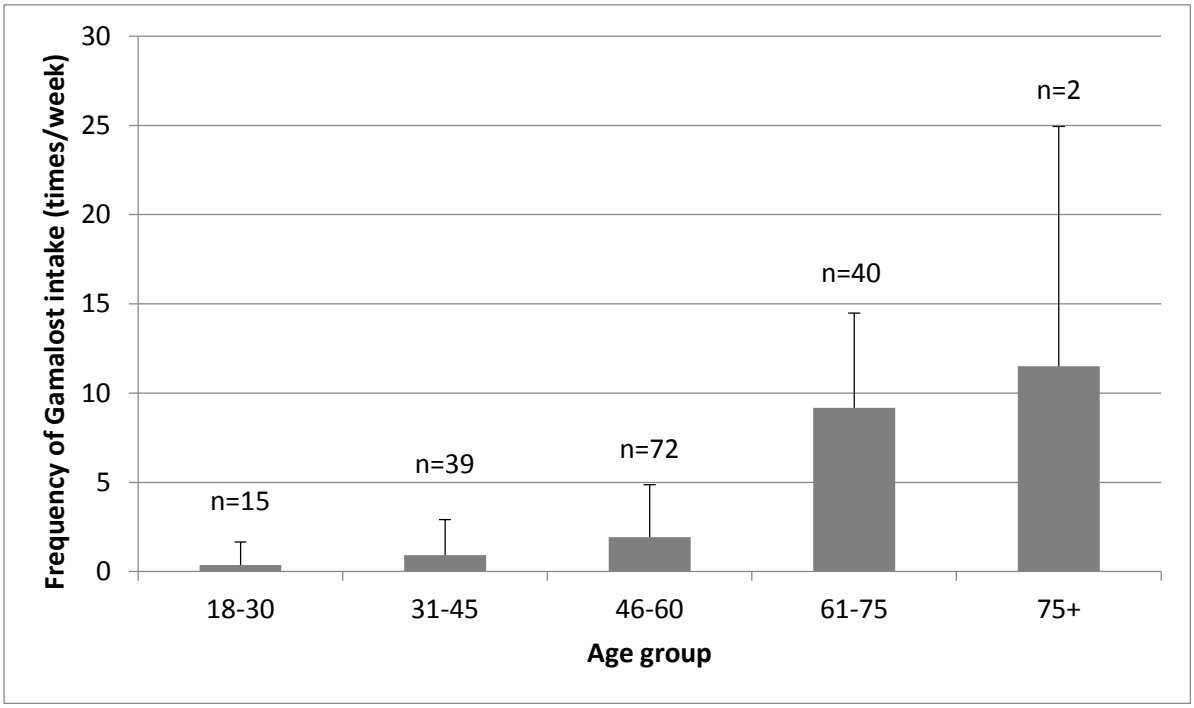

*Figure 3.1. Frequency of Gamalost intake related to the age groups of participants. From **paper I**. Mean weekly intakes of Gamalost (±SD) are 0.367 (1.29), 0.923 (1.99), 1.924 (2.95), 9.175 (5.31), and 11.5 (13.44), respectively for age groups one to five. Due to the large standard deviations, only the upper SD is shown in this figure.*

Due to this association, age was added as one of the confounding factors in the general linear model used to assess the relationship between Gamalost intake and BP, as well as other factors of the metabolic syndrome. The other potential confounders added in the statistical model were gender, education, waist circumference, physical activity, smoking habits, and total dairy product intake.

*Table 3.1. Crude and adjusted associations between Gamalost intake and blood pressure (from **paper I**).*

	Crude			Adjusted		
	B[2]	*P*	CI	B[2]	*P*	CI
SBP Gamalost[1]	0.179	0.609	-0.513, 0.872	-0.720	0.033*	-1.380, -0.059
DBP Gamalost[1]	0.095	0.682	-0.361, 0.551	-0.322	0.162	-0.776, 0.131

CI, confidence interval
[1]Frequency of intake: 0, 0.5, 2.0, 5.0, 10.5 and 21.0 times/week
[2]B represents the mean change in BP
*Indicates statistical significance, $P < 0.05$

The results on frequency of Gamalost intake and BP are presented in **table 3.1**. We found only a very small, but significant association of frequency of Gamalost intake on systolic BP. There are not many cross-sectional trials investigating the effect of cheese on BP, which makes comparisons difficult. Results from the cross-sectional Oslo Health Study showed frequency of all cheese intake to be significantly negatively associated with diastolic BP and borderline with systolic BP, with magnitudes smaller than the results from the current trial (B = -0.052, $P = 0.027$ and B = -0.061, $P = 0.079$ for diastolic and systolic BP, respectively) [29].

In addition to Gamalost intake, gender, age and waist circumference were also significantly associated with BP, as expected. Gamalost intake was not significantly associated with other factors of the metabolic syndrome, but was significantly and positively associated with total cholesterol (B = 0.068, $P = 0.01$), which was also reflected in LDL-cholesterol levels (B = 0.061, $P = 0.03$). Although these associations with cholesterol were very small, they were still surprising, as Gamalost does not contain any fat. Therefore, the association may be due some other factor that we were unable to distinguish in this cross-sectional trial. Despite the small associations found in this trial, it was an interesting finding and it was decided to build upon the research with an intervention trial.

3.3 Intervention trial on Gamalost and Gouda-type cheese intake and effect on metabolic syndrome variables

The second trial we conducted was a continuation of the cross-sectional trial, looking at similar exposures and outcomes, but with an intervention of cheeses (**papers II and III**). Due to the amount of work required and length of follow-up in this trial, it was conducted in Oslo

and Ås. As before, ethical approval was acquired from REK, who had full access to the study protocol, and the trial was added to the clinical trial registry clinicaltrials.gov. Some revisions were made to the questionnaire used in study 1, and other questionnaires were developed for the midway and end measurements. Like other similar trials, it was decided to do an intervention of moderate length, 8 weeks, in order to measure changes over time. Blood pressure was the main outcome, and was measured at inclusion, 4 weeks and 8 weeks. The other outcomes, cholesterol and metabolic syndrome variables, were measured at inclusion and end. A bioengineer was involved in the planning and implementation of the trial, ensuring proper handling of blood samples. Details on the study design, including the randomization procedure and outcome measures, can be found in **papers II and III**. Initially, we aimed to recruit 300 participants to the trial, 100 in each group. This proved difficult, likely due to many potential participants being hesitant towards consuming such frequent and large amounts of Gamalost. Setting the statistical power at 0.80 and criterion for significance at 0.05, yielded a sample size estimation of 53 cases per group and a total of 159, slightly higher than the 153 we were able to recruit in the end.

For the intervention trial, we wanted to investigate more than one exposure and outcome. In addition to Gamalost, the other exposure was to be a Gouda-type cheese, namely Norvegia. This cheese does not have a great ACE-inhibitory potential, but the *in vitro* digestion trial showed that the ACE-inhibitory activity increased greatly through digestion [118], making it an interesting addition regarding the effect on BP. Concerning the outcomes, in addition to BP, we wanted to investigate the effect of the cheeses on cholesterol level and variables of the metabolic syndrome. In **paper I**, frequency of Gamalost intake was significantly positively associated with cholesterol levels. Thus, we wanted to compare the effect of Gamalost, low in fat and calcium, with a cheese of regular fat and calcium content, since both fat and calcium intake is associated with cholesterol levels. The amount of the intervention cheeses to be consumed each day was decided based on protein content as well as ensuring the amount was higher than normal intake. Fifty grams of Gamalost and 80 g of Norvegia were determined to be the appropriate amounts for each group. Nutritional composition of the interventions is presented in **table 3.2**. Participants in the control group were asked to maintain their habitual diet, but to stay away from the two intervention cheeses and any similar cheeses.

They were given a list of cheeses they could consume whilst taking part in the trial, including cream cheese and blue cheeses.

*Table 3.2. Nutritional composition of intervention cheeses (adapted from **paper II**)*

Nutrient	Gamalost per 50 g	Norvegia per 80 g
Protein[a], g	25	22
Fat[a], g	0.5	22
Saturated, g	0	14
Calcium[a], mg	80	640
Sodium[a], mg	12	322
Magnesium[a], mg	6.5	26
Potassium[a], mg	49	62
IC_{50} ACE-inhibition[b]	0.34	0.59
ACE-inhibitory potential, mg[c]	0.12	0.02

[a]From TINE SA, manufacturer of Gamalost and Norvegia
[b]IC_{50} per unit weight of freeze-dried pH 4.6 soluble fraction (**SF**), expressed as mg pH 4.6 SF per ml. From Qureshi *et al,* 2012
[c]ACE-inhibitory potential, expressed as mg captopril equivalents per cheese weight. From Qureshi *et al,* 2012

As shown, the Norvegia intervention portion has a higher content of minerals, including calcium and magnesium, as well as a much higher fat content. Gamalost, on the other hand, has a slightly higher protein content than Norvegia. These two widely different cheeses could possibly have dissimilar effects on the variables of the metabolic syndrome, due to the different amounts of bioactive peptides, calcium and saturated fat. A higher amount of intervention cheese, like those used in Biong *et al.* (2004) [113] or Tholstrup *et al.* (2004) [112], would probably have made it easier to distinguish differences between the groups. However, it was determined that any higher intake would be too difficult for the participants to consume, especially in the Gamalost group because the cheese does not incorporate easily into cooking.

Some baseline characteristics of the three groups and the whole study population are presented in **table 3.3**. There were no significant differences between the groups at inclusion. Just over half the population were female, mean age 43 years, with a total cheese intake of approximately 7.5 servings/week.

*Table 3.3. Baseline characteristics (mean (SD[1]) or by %) of intervention study population (adapted from **papers II and III**).*

	Intervention group								
	All (*n*=153)		Gamalost (*n*=53)		Norvegia (*n*=50)		Control (*n*=50)		
Characteristic	Mean	SD	Mean	SD	Mean	SD	Mean	SD	*P*
Waist circumference (cm)	83.1	11.8	80.9	11.3	82.8	10.9	85.8	12.9	0.1
Systolic BP (mmHg)	132.3	17.2	131.5	19.3	130.6	14.7	134.8	17.2	0.4
Diastolic BP (mmHg)	82.4	9.8	82.5	10.6	81.4	8.9	83.1	10.0	0.7
Total cholesterol (mmol/L)	5.2	1.1	5.0	1.2	5.3	1.2	5.4	1.0	0.2
LDL cholesterol (mmol/L)	2.9	1.0	2.7	0.9	2.9	1.0	3.1	0.9	0.05
HDL cholesterol (mmol/L)	1.7	0.4	1.7	0.5	1.7	0.4	1.6	0.5	0.6
Triglycerides (mmol/L)	1.1	0.6	1.0	0.6	1.1	0.8	1.2	0.5	0.7
Blood glucose (mmol/L)	5.8	0.7	5.7	0.9	5.7	0.6	5.8	0.5	0.7
Metabolic syndrome (%)	30.1		24.5		32.0		34.0		0.5
Total dairy[7]	18.4	11.9	19.7	12.9	17.5	10.1	18.0	12.7	0.6
Total cheese[7]	7.5	4.6	8.0	4.7	7.1	4.2	7.2	4.9	0.5
Gouda-type cheese[7]	5.7	4.3	6.1	4.6	5.4	3.6	5.6	4.8	0.7
Gamalost[7]	0.7	1.9	0.6	1.7	0.6	1.8	0.8	2.2	0.9

[1]SD, standard deviation

[2]Percentage who have either SBP>140, or DBP>90

[3]Percentage daily smokers

[4]Percentage who reported moderate to hard physical activity more than four hours per week

[5]Percentage who salt their food

[6]Percentage who consume alcohol >1/week

[7]Servings per week

The first paper from this trial focused on the effect of the cheeses, especially Gamalost, on blood pressure (**paper II**). It was hypothesised that, due to the high amount of ACE-inhibiting peptides in Gamalost cheese, BP would decrease in this group compared to control. The BP measurements were carried out according to the American Heart Association recommendations [119], i.e. after a 10 minute rest and in a relaxed seated position. In addition to the standard BP measurements, it was attempted to measure amount of ACE in blood serum, as described in Karlsen *et al.* (2013) [120]. The ACE kinetic kit, manufactured by Bühlmann Laboratories AG, Switzerland, was easy to use but the results were deemed unsatisfactory. The variations between the replicates were simply too large within the samples and too inconsistent between the samples, hence it was not possible to draw any conclusions from those results.

From baseline to the end of the trial, systolic BP decreased in all the three intervention groups, whereas diastolic BP only decreased significantly in the Gamalost group (paired samples t-test). Intention-to-treat analysis of the cheese groups compared to the control showed no effect of the interventions on BP (Dunnett test). The participants within each intervention group were also stratified by their BP level at baseline, to assess whether there were different responses to the interventions depending on initial BP. Even though there are no cheese intervention trials to compare to, similar trials on dairy products indicate that significant changes in BP are more likely to occur in populations with higher BP. Consequently, participants were categorized as described in **table 3.4**.

*Table 3.4. Blood pressure categories (from **paper II**)*

Category	Blood pressure (mmHg)
Optimal	Systolic <120 and diastolic < 80
Normal-high	Systolic 120-139 and/or diastolic 80-89
Hypertensive	Systolic ≥ 140 and/or diastolic ≥ 90

Figure 3.2 A and B illustrates BP changes through the trial in each intervention group, separated by BP category at baseline. There were no BP changes in those participants who had optimal BP at baseline. At the end of the trial, systolic BP was reduced for hypertensive participants in the Gamalost and control groups, whereas diastolic BP was only reduced in the Gamalost group (paired samples t-test). In the subgroup with normal-high BP, systolic BP was

reduced in the Norvegia group and diastolic BP was reduced in the Gamalost group. When comparing the mean BP change in the intervention groups with the control group (Dunnett test), there were no significant reductions in BP. At 4 weeks, the normal-high BP participants in the Gamalost group had borderline significantly lower BP than the control group (-3.5 mmHg, $p = 0.08$). Also noteworthy, even though systolic BP was reduced in the hypertensive subgroup in the Norvegia intervention, compared to the reduction in the control group, BP was significantly higher in the Norvegia group (10.5 mmHg, $p = 0.03$). Similar trials on other dairy products have shown mixed results, as discussed in section 1.7.2 of this thesis. There seems to be some influence of baseline BP level, as a trial involving tablets with fermented milk found significantly lower diastolic BP compared to control in those who had normal-high BP at baseline (-5.0 mmHg, $p = 0.045$), but in the hypertensive subgroup systolic BP was significantly lower (-11.2 mmHg, $p = 0.003$) [109]. However, in that trial, the intervention lasted only 4 weeks, so it is not known whether this reduction would be maintained for a longer period. Another trial, investigating different forms of the lactotripeptides VPP and IPP, found no effect of the interventions compared to control at 8 weeks of intervention in normal-high BP or hypertensive subjects [80].

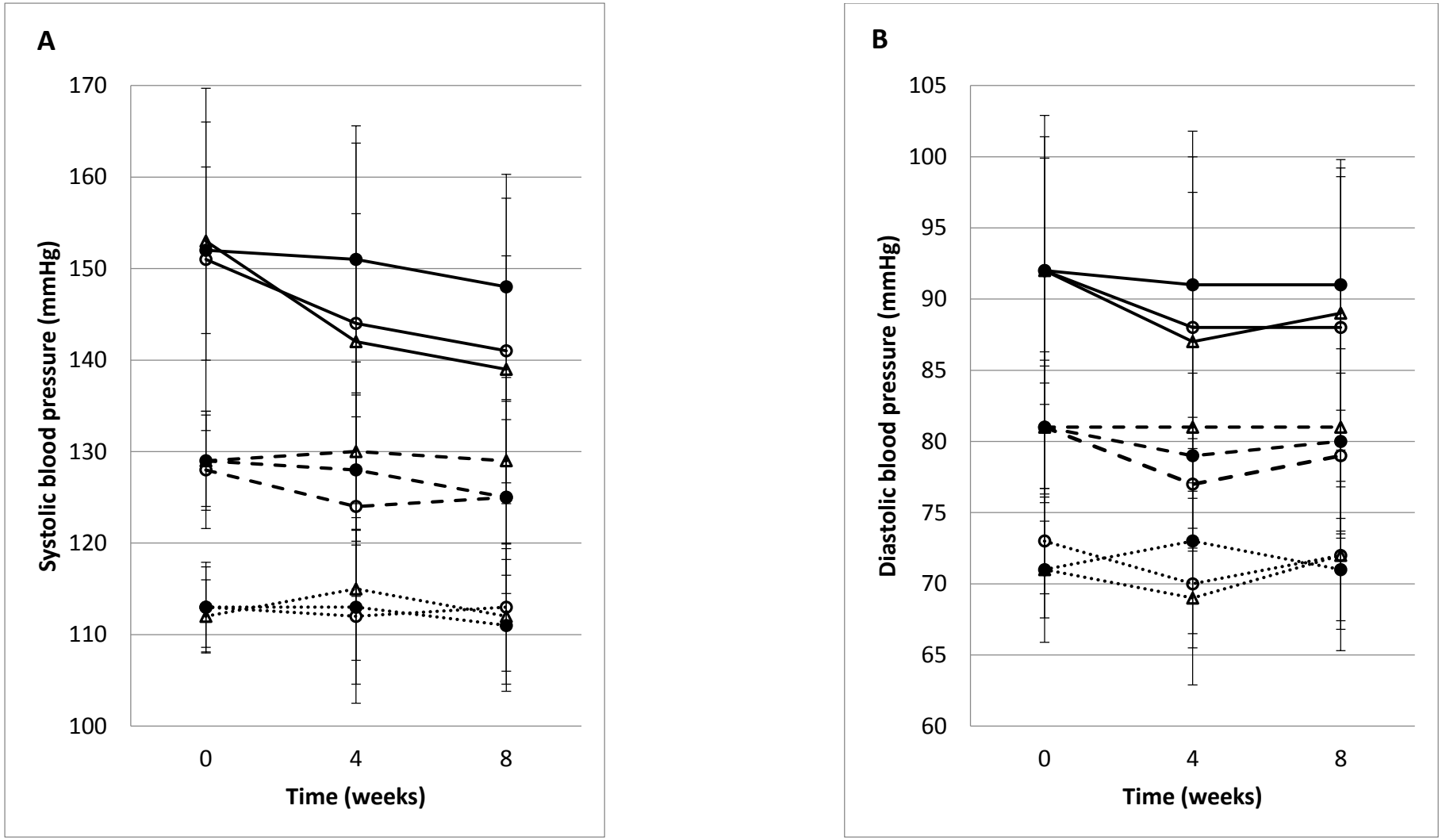

*Figure 3.2. Systolic (A) and diastolic (B) blood pressure (mean (SD)) at inclusion, midway and end, in three intervention groups. From **paper II**. Solid lines: participants with hypertension at inclusion; dashed lines: participants with normal-high BP at inclusion, and dotted lines: participants with optimal BP at inclusion. ○Gamalost, ●Norvegia, ⊿Control*

The second paper of this trial focused on the effect of cheeses, with differences in the content of fat, calcium and bioactive peptides, on changes in cholesterol and variables of the metabolic syndrome (**paper III**). As presented in the theory section of this work, cheese is not usually recommended as part of heart healthy diets. However, recent evidence suggest cheese may not be as atherogenic as previously thought. It was hypothesised that cholesterol would not increase and variables of the metabolic syndrome would not change due to the high intake of cheese in the trial. The measurements were taken at inclusion and at the end of the trial (week 8). Again, the participants were stratified based on metabolic syndrome at baseline (metS-yes or metS-no), as well as by individual variables of the metabolic syndrome. Total and LDL-cholesterol are not variables of the metabolic syndrome and were therefore stratified based on the cholesterol guidelines from "Adult Treatment Panel III" [121]. Hence, participants were stratified as LDL-yes/LDL-no with cut-off at 3.4 mmol/L and CHOL-yes/CHOL-no with cut-off at 5.2 mmol/L.

*Table 3.5. Stratified analysis by MetS-yes or by total cholesterol-yes, comparing control group with the two cheese diets. Values are mean (SE), 2-sided p-values for the difference from control (Dunnett test). Adapted from **paper III**.*

	Baseline mean	End mean	Change	Difference from control (95% CI)	*p*
MetS-yes Tot chol (mmol/L)					
Control	5.45 (0.23)	5.41 (0.29)	0.11 (0.22)		
Norvegia	6.01 (0.33)	5.49 (0.29)	-0.59 (0.13)	-0.70 (-1.25, -0.14)	0.013
Gamalost	5.25 (0.33)	5.22 (0.22)	-0.03 (0.15)	-0.14 (-0.72, 0.44)	0.813
Tot chol-yes Tot chol (mmol/L)					
Control	6.06 (0.14)	6.00 (0.17)	0.01 (0.11)		
Norvegia	6.03 (0.19)	5.66 (0.20)	-0.39 (0.11)	-0.39 (-0.73, -0.05)	0.021
Gamalost	6.11 (0.18)	5.75 (0.14)	-0.39 (0.10)	-0.40 (-0.77, 0.02)	0.035

At the end of the trial, there were no increases in cholesterol levels in any of the intervention groups. The paired samples t-test showed that total cholesterol was significantly reduced in the Norvegia group from baseline to the end of the trial (-0.2 mmol/L, $p = 0.017$). As shown in **table 3.5**, when stratifying based on baseline values, and comparing with the control group (Dunnett test), it was found that for those participants who had metabolic syndrome, mean

total cholesterol change was significantly reduced compared to control (-0.7 mmol/L, p = 0.013). For those participants who had high total cholesterol at baseline, mean cholesterol change was significantly reduced in both the Norvegia and the Gamalost groups compared to control. There were no significant changes in LDL- or HDL- cholesterol. Regarding other variables of the metabolic syndrome, waist circumference was significantly reduced in the Gamalost group compared to control, and serum triglyceride level was reduced in the Norvegia group compared to control (-0.7 mmol/L, p = 0.047) in those participants who had metabolic syndrome at baseline. There were no significant effects of the cheese interventions on other metabolic syndrome variables when comparing with the control group.

Our results on cholesterol in this trial support the hypothesis that cheese, despite the high saturated fat content in many of them, may have a neutral or even positive effect on cholesterol levels and variables of the metabolic syndrome. These results are in accordance with other intervention trials such as Tholstrup *et al.* 2004 [112], as well as results from observational trials such as the Oslo Health Study [29] and the Observation of Cardiovascular Risk Factors in Luxembourg study [96]. As presented in sections 1.6.3 and 1.7.3 of this thesis, the seemingly hypocholesteremic effect of cheese may be due to a number of mechanisms. Some of the suggested factors include calcium, bioactive peptides, or specific fatty acids such as conjugated linoleic acid (CLA). As the results from the current work show, we still have no clear understanding of why a high cheese intake does not raise serum cholesterol levels. Both intake of Gamalost, fat free and low in calcium, and Norvegia, 28% fat and rich in calcium, were associated with reductions in total cholesterol in those participants who had high cholesterol at baseline, whereas only the Norvegia intervention provided reductions in total cholesterol for those participants who had metabolic syndrome. The ACE-inhibiting peptides have received the most attention in research, and only the ACE-inhibitory activity of bioactive peptides have been characterized in Norvegia and Gamalost. This means we cannot rule out that there are other bioactive peptides which may be hypocholesteremic, but we cannot know for certain at this time. One suggested mechanism for the hypocholesteremic effect of cheese is through inhibition of Δ9-desaturase activity by some unidentified cheese components, possibly related to CLA [122]. However, as Gamalost does not contain any CLA, this is likely not the only explanation for the hypocholesteremic effect of the cheeses in the current trial.

3.4 Strengths and limitations of the trials

As with all trials, especially involving human subjects, there are some limitations to these two trials that should be addressed. Firstly, cross-sectional trials, especially with such a small sample size, cannot provide sufficient evidence for a causative relationship between the exposure and the outcome. However, cross-sectional trials can give an overview of an interesting association and pinpoint where future research is needed. The limitations of the intervention trial are presented in paper II. In brief, using in office BP measurements may have provided less accurate results than if all the participants had 24-hour ambulatory BP measurements. Also, mean BP in the population was only moderately high, meaning the results may not be relevant for populations with higher BP. Since the population was free-living, we did not have complete control of the participants' diets.

The main strength of the trials is that we specifically investigated the effect of particular cheeses on health variables, as opposed to investigating the generic term "cheese" or "dairy products". Both of the study samples were relatively homogenous, with similar ethnicities and dietary habits. The intervention trial had a long duration and retention of the participants was very good, with only five subjects lost to follow-up.

4. MAIN CONCLUSIONS AND FUTURE PERSPECTIVES

Based on the results from the current study, it is concluded that:

- Gamalost intake is higher in older age groups.

- Cross-sectional results showed higher frequency of intake of a cheese rich in ACE-inhibiting peptides, such as Gamalost, to be associated with slightly reduced systolic blood pressure.

- An intervention of a high intake of a Gouda-type cheese did not adversely affect any variables of the metabolic syndrome.

- Compared to a control group of low cheese intake, Gouda-type cheese intake was associated with lower serum cholesterol in participants with high cholesterol and metabolic syndrome.

- A high intake of a cheese rich in ACE-inhibiting peptides was borderline significantly associated with reduced diastolic blood pressure after four weeks of intervention, for those participants who had normal-high blood pressure. This association was less significant at eight weeks.

- The current study does not provide enough data yet to support that ACE-inhibiting peptides in cheese can lower blood pressure.

- Our results add to the findings that a high intake of saturated fat from cheese may not raise cholesterol.

To add knowledge to the existing literature, and to further add to the observations obtained in this work it is suggested to further investigate the following topics:

- Dose-response of Gamalost

  Participants in an intervention trial could consume varying servings of Gamalost, and post-meal blood pressure development would be assessed over 120 minutes.

- The underlying molecular mechanisms of action of how the ACE-inhibiting peptides identified in dairy products actually inhibit the enzyme.

- Perform similar trials and compare with other dairy products rich in ACE-inhibiting bioactive peptides.

- Investigate which cheeses and other dairy products might be hypocholesteremic. What are mechanisms which can contribute to the hypocholesteremic effect? Proteins and peptides should be investigated further, as the results from the current trial do not support that calcium or fat alone are responsible for the effect.

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6. PAPERS I-III

PAPER I

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Short communication: Is consumption of a cheese rich in angiotensin-converting enzyme-inhibiting peptides, such as the Norwegian cheese Gamalost, associated with reduced blood pressure?

R. Nilsen,*[1] A. H. Pripp,† A. T. Høstmark,‡ A. Haug,§ and S. Skeie*
*Department of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences, PO Box 5003, N-1432 Aas, Norway
†Department of Biostatistics, Epidemiology and Health Economics, Oslo University Hospital, N-0450 Oslo, Norway
‡Institute of Health and Society, University of Oslo, N-0450 Oslo, Norway
§Department of Animal and Aquacultural Sciences, Norwegian University of Life Sciences, N-1432 Ås, Norway

ABSTRACT

Epidemiological and clinical studies have shown that angiotensin-converting enzyme (ACE)-inhibiting peptides derived from dairy products may decrease blood pressure. These peptides have been identified in many cheeses, and Gamalost, a traditional Norwegian cheese, is particularly rich in these peptides. The aim of this cross-sectional study was to examine whether frequency of Gamalost intake was associated with blood pressure in a Norwegian population sample. Blood pressure and other clinical measurements, including the factors of metabolic syndrome, were obtained from 168 participants (56% female, mean age = 51 yr) who completed a questionnaire about dietary habits and other health-related factors. Mean Gamalost intake was 2 servings per week. The prevalence of hypertension was 23.8% in the population, with mean systolic and diastolic blood pressures of 128 and 78 mmHg, respectively. Intake of Gamalost was inversely associated with systolic blood pressure. Each increase in frequency unit of Gamalost intake corresponded to a reduction in systolic blood pressure of 0.72 mmHg, after controlling for sex, age, education, waist circumference, physical activity, smoking status, and dairy food intake. Results from this study indicate that consumption of Gamalost (or other foods rich in ACE-inhibiting peptides) may reduce blood pressure.

Key words: cheese, angiotensin-converting enzyme (ACE)-inhibiting peptide, blood pressure, dairy product

Short Communication

Milk proteins are considered one of the most important sources of bioactive peptides (Korhonen and Pihlanto, 2006) and studies have found that different cheeses contain several bioactive peptides in varying amounts. Angiotensin-converting enzyme (**ACE**) is an important enzyme in the renin-angiotensin system, which is one of the pathways that control blood pressure. The effect of ACE is to activate angiotensin II, a vasoconstrictor, and inactivate bradykinin, a vasodilator (Silva and Malcata, 2005; FitzGerald et al., 2004), resulting in an increase in blood pressure. Peptides with ACE-inhibiting or blood pressure (**BP**)-lowering activity have been identified in many cheeses (Sieber et al., 2010). Cheese and other dairy products are significant sources of saturated fat in the typical western diet (Sonestedt et al., 2011), a fat that may increase the amount of low density lipoprotein (**LDL**) cholesterol in the blood, which is a risk factor for cardiovascular disease (**CVD**). However, some studies have found that a higher intake of dairy products is associated with a reduced risk of CVD, and it was recently found that cheese intake is negatively associated with the metabolic syndrome (Høstmark and Tomten, 2011). Part of the reason why cheese may be protective against CVD could be the presence of bioactive peptides.

Gamalost is an autochthonous Norwegian cheese that is naturally low in fat (<1%), does not contain salt, and is high in protein (50%). Details on the production and ripening of Gamalost have been described elsewhere (Qureshi et al., 2012). The cheese was found to have a higher ACE-inhibitory potential than Norvegia, a Gouda-type cheese (Pripp et al., 2006; Qureshi et al., 2012, 2013), and it is one of the cheeses with the highest ACE-inhibitory potential (Sieber et al., 2010). Even though ACE-inhibiting peptides have been found in many cheeses, few studies describe their effect in humans.

The Global Burden of Disease Study 2010 identified high BP as the leading risk factor for global disease burden (Lim et al., 2012). Hypertension is a major risk factor for CVD, and systolic BP >130 mmHg or diastolic BP >85 mmHg are 2 of the diagnostic criteria

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[1]Corresponding author: rita.nilsen@nmbu.no

for metabolic syndrome. Cardiovascular disease is the most common cause of death in Norway, accounting for about 35% of all deaths (Folkehelseinstituttet, 2010). The prevalence of hypertension in the adult population in the United States is about 30% (Yoon et al., 2010), and it has been estimated that a decrease in diastolic BP of just 5 mmHg can reduce the risk of CVD by 16% (FitzGerald et al., 2004). Pharmacological treatment of hypertension is often associated with undesirable side effects such as reduced kidney function and hypotension (Haque and Chand, 2008). Consequently, food-derived ACE-inhibitors would be of great interest, as these are not associated with side effects. A meta-analysis of randomized controlled trials on the effect of food-derived peptides on BP found a significant reduction in both systolic and diastolic BP, indicating a possible role for food in the management of mild hypertension (Pripp, 2008). We are not aware of any published observational studies with clinical tests regarding the association between cheese intake and BP. The aim of this epidemiological study was to assess whether the frequency of Gamalost intake was associated with blood pressure and other factors of the metabolic syndrome, in the population of Vik i Sogn, a small community on the Norwegian west coast.

This cross-sectional study was conducted in Vik, Norway, in May 2012. The adult population of Vik comprised the study sample. Participants were recruited through the 4 largest work places in Vik, and one person worked specifically to reach the elderly population. Furthermore, a short article was published in the local newspaper inviting people to participate in the study. One hundred eighty-six people completed the questionnaire. Of those, 5 did not show up for clinical assessment. Pregnant women and participants lacking information on cheese and dairy intake were excluded from the analyses, resulting in a final study sample of 168. Subjects who lacked information on the factors included in the ANOVA or who reported taking BP-lowering medications were further excluded from this analysis, resulting in a sample size of 153. This study was conducted according to the guidelines in the Declaration of Helsinki, and all procedures involving human subjects were approved by Regional Committees for Medical and Health Research Ethics (Oslo, Norway) on April 24, 2012. Written informed consent was obtained from all subjects. The participants were offered breakfast and one Gamalost cheese as compensation for participation.

A questionnaire was developed specifically for this study, based on the previously used food frequency questionnaire from the Oslo Health Study (Mostøl, 2004). In addition to questions about health and physical activity, the questionnaire included a short section on dietary habits, emphasizing dairy intake. Four questions inquired about the intake of cheese, including all cheese, regular (mostly Gouda type) cheeses, brown whey cheese, and Gamalost. Five questions inquired about the intake of other dairy products; for example, milk and yogurt. The variables concerning cheese intake were categorized into rarely or never, 1 to 3 times per month, 1 to 3 times per week, 4 to 6 times per week, 1 to 2 times per day, and 3 times or more per day. For statistical analyses, the midpoint in each category was recalculated into frequency in times per week; that is, 0, 0.5, 2.0, 5.0, 10.5, and 21.0 servings per week, respectively. Total dairy product intake was calculated by summarizing the frequency of intake of all cheese, all milk, and fermented milk.

Height was measured to the nearest 0.1 cm using a portable stadiometer (Seca 217, Seca, Hamburg, Germany). Weight was measured to the nearest 0.1 kg using digital scales (TBF-300A Body Composition Analyzer, Tanita, Tokyo, Japan). Waist circumference was measured in accordance with World Health Organization recommendations, at the midpoint between the iliac crest and the lowest rib margin, to the nearest 0.1 cm (WHO, 2011) using a measuring tape (Seca 201 Circumference measuring tape, Seca). Body mass index (BMI) was calculated as weight (kg) divided by the square of height (m).

Blood pressure was measured according to recommendations from the American Heart Association (Pickering et al., 2005). Participants rested for approximately 10 min before BP was measured using a Microlife BP A200 BP meter (Microlife, Widnau, Switzerland). Three consecutive measurements were taken, and the average of the second and third measurements was used for analysis (automatically calculated by the blood pressure device). In some cases, the device used 4 measurements to get a more accurate reading. Venous blood samples were drawn in the morning after an overnight fast (approximately 10–12 h), using the Vacutainer system (Becton Dickinson Co., Franklin Lakes, NJ). The samples were centrifuged at 833 $\times$ g for 10 min at room temperature, and the serum was separated 1 to 2 h after the blood was drawn. The serum was frozen to −20°C within 5 h. Fürst Medical Laboratories (Oslo, Norway) conducted the lipid analyses. The measured biochemical markers were total cholesterol, high density lipoprotein (**HDL**) cholesterol, LDL cholesterol, and triglycerides. Blood glucose was measured in capillary blood by the finger stick method.

Daily physical activity was assessed by 2 questions in the questionnaire; one question regarding amount of leisure time physical activity and one regarding type of physical activity. For statistical analyses, participants were classified into 3 groups of physical activity: sed-

entary, light physical activity, and moderate to hard physical activity. Use of tobacco products was self-reported, and classified into currently using, previously used, and never used.

The SPSS 19.0 software package (IBM Corp., Armonk, NY) was used for the statistical analyses. When appropriate, one-way ANOVA was used to evaluate significance of differences of mean values between the groups of Gamalost intake frequency, using Bonferroni correction for multiple comparisons (Table 1). The statistical significance level was set at $P < 0.05$. The independent samples t-test was used to assess the difference in means between men and women. Age in years was used as a continuous variable in most analyses, except when regarding the frequency of Gamalost intake (Figure 1), where the participants were grouped into 5 age groups: 18 to 30 yr, 31 to 45 yr, 46 to 60 yr, 61 to 75 yr, and 76+ yr. This was done because of the study sample size and the wide range of ages (61 yr). General linear models were used to assess the association between intake frequency of Gamalost and selected outcomes (systolic and diastolic BP, blood glucose, serum triglycerides, and serum cholesterol), which included adjustment for several potential confounders. These potential confounders were chosen because they are likely to have an effect on BP and could be associated with intake of Gamalost. Several of them are important factors in the treatment and control of hypertension (Krousel-Wood et al., 2004). The adjusted model included 8 factors: sex, age in years, education (total years in school), waist circumference (cm), physical activity (4 levels: sedentary, light physical activity, moderately hard physical activity, hard physical activity several times per week), smoking (currently, previously, never) and total dairy product intake (6 intake levels, as described above).

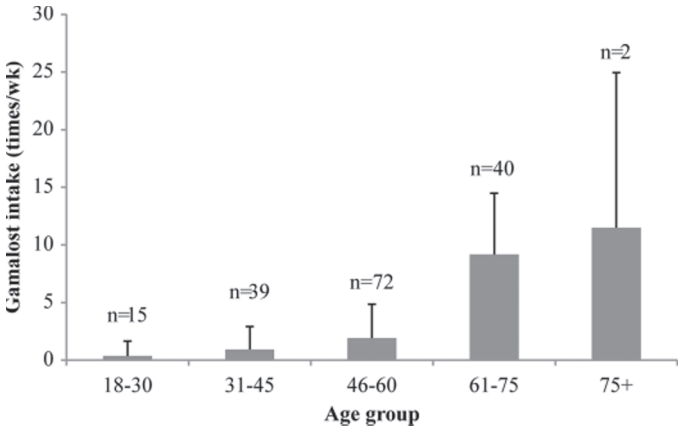

Figure 1. Frequency of Gamalost intake related to the age groups of participants. Mean weekly intakes (times/wk) of Gamalost (±SD) were 0.367 (1.29), 0.923 (1.99), 1.924 (2.95), 9.175 (5.31), and 11.5 (13.44), respectively, for age groups 1 through 5. Due to the large standard deviations, only the upper standard deviation is shown in this figure.

Some characteristics of the study sample are shown in Table 1, according to intake frequency of Gamalost (times/wk). Sex, age, waist circumference, education, intake of all cheese, intake of regular cheese, and total dairy product intake were all significantly different between the 6 levels of Gamalost intake frequency. Both

Table 1. Some sample characteristics [mean (SD) or %] by frequency of Gamalost intake

	Gamalost intake frequency (servings/wk)							
Item	2.2 (n = 168) Sample total	0 (n = 60)	0.5 (n = 45)	2.0 (n = 29)	5.0 (n = 18)	10.5 (n = 13)	21.0 (n = 3)	P-value[1]
Sex (% female)	56.0	66.7	46.7	65.5	55.6	23.1	33.3	0.04
Age (yr)	50.8 (13.2)	43.4 (13.1)	51.8 (11.0)	54.6 (10.3)	58.2 (13.6)	57.8 (8.2)	71.0 (8.7)	<0.001
Weight (kg)	76.3 (13.7)	74.5 (14.7)	77.1 (13.8)	75.4 (10.3)	77.9 (14.5)	81.0 (15.2)	80.7 (8.3)	0.63
Height (cm)	172.8 (8.9)	171.3 (9.2)	173.7 (8.0)	172.6 (8.3)	172.9 (10.5)	178.5 (8.3)	166.5 (6.9)	0.10
Waist circumference (cm)	85.7 (11.1)	82.5 (10.6)	86.8 (12.0)	84.9 (8.6)	88.0 (9.9)	91.5 (11.3)	104.1 (12.8)	0.002
Body mass index (kg/m^2)	25.5 (3.5)	25.2 (3.5)	25.5 (3.8)	25.3 (3.0)	26.0 (3.5)	25.3 (3.4)	29.3 (4.7)	0.52
Systolic blood pressure (mmHg)	128.0 (15.1)	124.3 (12.8)	131.9 (18.1)	127.9 (17.2)	127.1 (11.2)	130.1 (11.7)	138.3 (6.8)	0.14
Diastolic blood pressure (mmHg)	77.7 (9.9)	76.1 (8.8)	79.7 (11.4)	76.5 (8.9)	77.5 (11.0)	81.1 (9.9)	77.7 (7.0)	0.39
Hypertension[2] (%)	23.8	15.0	26.7	31.0	22.2	38.5	33.3	0.38
Education (yr)	14.0 (3.2)	15.0 (3.1)	13.6 (2.9)	13.8 (2.8)	12.5 (4.4)	13.2 (3.4)	12.0 (4.2)	0.05
Smoking[3] (%)	10.7	5.0	15.6	13.8	0.0	30.8	0.0	0.10
Physical activity[4] (%)	34.1	41.4	25.0	37.9	23.5	38.5	33.3	0.53
Servings/wk								
All cheese	8.7 (5.2)	6.5 (4.8)	8.6 (5.1)	9.5 (4.3)	10.4 (4.5)	11.7 (4.4)	21.0 (0.0)	<0.001
Regular cheese	5.9 (4.5)	5.1 (4.5)	5.3 (4.4)	6.2 (3.4)	7.0 (3.8)	7.7 (5.3)	15.8 (7.4)	0.007
Total dairy	22.1 (11.8)	19.6 (11.9)	20.0 (8.7)	23.5 (12.6)	25.3 (11.5)	30.5 (11.7)	45.5 (14.8)	0.001

[1]Difference in characteristics between groups (one-way ANOVA test); $P < 0.05$.

[2]Percentage who have systolic blood pressure >140 mmHg, diastolic blood pressure >90 mmHg, or both.

[3]Percentage of daily smokers.

[4]Percentage who reported moderate to hard physical activity daily.

Table 2. Crude and adjusted associations between 8 selected factors and systolic blood pressure (n = 153)

	Crude			Adjusted		
Item	B[1]	*P*-value	95% CI	B	*P*-value	95% CI
Gamalost intake[2]	0.179	0.61	−0.513, 0.872	−0.720	0.03*	−1.380, −0.059
Sex, if male	10.481	<0.001*	6.113, 14.849	5.988	0.02*	0.973, 11.003
Age	0.397	<0.001*	0.232, 0.562	0.311	0.001*	0.130, 0.492
Years of education	−0.685	0.07	−1.415, 0.045	−0.169	0.63	−0.863, 0.525
Waist circumference	0.674	<0.001*	0.485, 0.864	0.436	<0.001*	0.194, 0.678
Physical activity, if moderate to hard	2.469	0.36	−2.869, 7.807	2.901	0.20	−1.595, 7.396
Smoking, if no	0.014	0.99	−3.460, 3.488	−0.729	0.65	−3.919, 2.460
Total dairy intake[2]	0.050	0.63	−0.152, 0.251	0.028	0.78	−0.165, 0.221

[1]B represents the mean change in blood pressure.

[2]Frequency of intake: 0, 0.5, 2.0, 5.0, 10.5 and 21.0 times/wk.

*$P < 0.05$.

age and waist circumference were highest for the 3 highest intake levels of Gamalost. The mean intake frequency of Gamalost ranged from 0.37 to 11.5 times/wk in the youngest and oldest age groups, respectively. Intake of Gamalost and age showed a significant positive correlation, r = 0.37 ($P < 0.001$, 2-tailed), as expected from Norwegian tradition. For illustration, the mean intake frequency of Gamalost in different age groups is shown in Figure 1. Mean intake of Gamalost was significantly higher in men (mean = 2.96 servings/wk; SD = 4.66) than in women (mean = 1.61; SD = 3.04), $P = 0.03$ (2-tailed; *t*-test). The intakes of other cheeses and total dairy products closely followed the intakes of Gamalost, with the highest intakes being in the highest intake levels of Gamalost. The prevalence of hypertension in the total population was 23.8% (crude estimate) and was not significantly different between the 6 levels of Gamalost intake.

Gamalost Intake and Blood Pressure

Cross-sectional associations between 8 selected factors and systolic BP are presented in Table 2. The crude model shows that only sex, age, and waist circumference were associated with systolic BP. An adjusted model was made to control for potential confounders. The multivariable adjusted model showed a statistically significant inverse association between frequency of intake of Gamalost, and systolic BP [unstandardized regression coefficient used in the general linear model (**B**) = −0.72, $P = 0.03$]. The results show that for each increase in the intake frequency unit of Gamalost (times/wk), systolic BP decreased by 0.72 mmHg. Despite the smaller sample size, this is comparable to what has been found in larger population-based studies on cheese consumption (Høstmark and Tomten, 2011; Sonestedt et al., 2011). The prevalence of hypertension, defined as having a systolic BP >140 or diastolic BP >90 (Chobanian et al., 2003), varied greatly between the subjects in each of the intake levels of Gamalost. The lowest prevalence of hypertension was found in the group that did not consume Gamalost. However, we also observed a significant difference in age between the participants in the 6 levels of Gamalost intake, with the nonconsumers also being the youngest group. It is well established that BP increases with age (Whelton et al., 2002; Wolf-Maier et al., 2003), which can partly explain why BP was, in the crude univariable statistical analyses, associated positively with intake of Gamalost and, thus, the need to use multivariable statistical models to adjust for possible confounders. The adjusted model also showed that sex (being male), age, and waist circumference were still significantly associated with systolic BP. As shown in Table 3, a nonsignificant inverse association was found between Gamalost intake and diastolic BP (B = −0.322, $P = 0.16$). As with systolic BP, age, sex, and waist circumference were significantly associated with diastolic BP in the crude model. In the adjusted model, only waist circumference was significantly associated with diastolic BP. This is in accordance with a similar but larger study, which found that a higher frequency of cheese intake was borderline significantly associated with a reduction in systolic BP [standardized regression coefficient (β) = −0.33, $P_{trend} = 0.06$] but not with diastolic BP (Sonestedt et al., 2011). Education, physical activity, smoking, or total dairy intake had no effect on either systolic BP or diastolic BP. Although diastolic BP does not increase after the age of 50, systolic BP continues to increase throughout life (Chobanian et al., 2003), making management of systolic hypertension very important in the older population. Estimations show that only a 2 mmHg decrease in systolic BP in a population would reduce mortality from stroke by 6%, whereas a 5 mmHg reduction would reduce mortality from stroke by as much as 14% (Whelton et al., 2002).

Table 3. Crude and adjusted associations between 8 selected factors and diastolic blood pressure (n = 153)

	Crude			Adjusted		
Item	B[1]	*P*-value	95% CI	B	*P*-value	95% CI
Gamalost intake[2]	0.095	0.68	−0.361, 0.551	−0.322	0.16	−0.776, 0.131
Sex, if male	5.951	<0.001*	2.723, 9.180	2.209	0.21	−1.232, 5.650
Age	0.172	0.009*	0.043, 0.301	0.093	0.14	−0.031, 0.218
Years of education	−0.273	0.33	−0.830, 0.284	0.059	0.81	−0.417, 0.536
Waist circumference	0.432	<0.001*	0.299, 0.564	0.393	<0.001*	0.227, 0.558
Physical activity, if moderate to hard	−0.046	0.98	−3.631, 3.539	−0.174	0.91	−3.259, 2.911
Smoking, if no	−0.155	0.90	−2.596, 2.285	−0.885	0.43	−3.073, 1.304
Total dairy intake[2]	−0.011	0.87	−0.144, 0.122	−0.023	0.73	−0.156, 0.109

[1]B represents the mean change in blood pressure.

[2]Frequency of intake: 0, 0.5, 2.0, 5.0, 10.5, and 21.0 times/wk.

*$P < 0.05$.

If it is confirmed that an increased intake of Gamalost is associated with reduced systolic BP, it is important to establish whether the association is causal and of clinical significance. Provided that the observed association is causal, it would appear that increased intake of a cheese with a high ACE inhibitory activity, such as Gamalost, to the levels seen in the highest intake frequency group in this population might reduce BP, and thus mortality, in a population. Although some studies on the effect of milk-derived bioactive peptides on hypertension have shown an effect in normotensive subjects, most successful intervention trials have been on subjects with mild or moderate hypertension (Engberink et al., 2008). It is tempting to speculate that we would find a larger effect of Gamalost on BP if more participants in the study had hypertension.

In addition to BP, other factors of the metabolic syndrome were included in this study. Table 4 shows the significant associations in the adjusted model for these factors. Nonsignificant associations were excluded from this table. As can be seen from the table, increased waist circumference and smoking were both positively associated with increased blood glucose and triglycerides. Furthermore, serum HDL level was significantly negatively associated with being male (B = −0.244, $P = 0.001$) and inversely associated with waist circumference (B = −0.014, $P < 0.001$). With increasing intake frequency of Gamalost, a significant increase in total serum cholesterol (B = 0.068, $P = 0.01$) occurred, which was also reflected in LDL cholesterol (B = 0.061, $P = 0.03$) but not HDL cholesterol.

Results on the role of cheese in the etiology of different CVD have been contradictory. Most studies on this subject are on the role of dairy products, with cheese comprising only a small subsection of the study. Furthermore, the CVD umbrella can be very unspecific, with the outcome measures being very different from study to study. Moreover, the nutritional composition of cheese is highly variable, making it difficult to generalize results. A recent meta-analysis of prospective cohort studies on the role of dairy in hypertension found that per 200 g/d of total dairy, the risk of hypertension was lowered by 3% (risk ratio: 0.97, 95% CI: 0.95, 0.99; Soedamah-Muthu et al., 2012). However, the same meta-analysis of 51,007 individuals with mean cheese intakes ranging from 10 to 43 g/d showed no effect of cheese intake on hypertension (risk ratio = 1.00, 95% CI: 0.98, 1.03). This might, however, reflect the large variation in the amount and activity of bioactive peptides in different cheeses and dairy products.

Table 4. Adjusted associations between selected factors and blood glucose, triglycerides, and cholesterol (n = 153)[1]

	Blood glucose		Cholesterol		Triglycerides		High-density lipoprotein cholesterol		Low-density lipoprotein cholesterol	
Item	B[2]	*P*-value	B	*P*-value	B	*P*-value	B	*P*-value	B	*P*-value
Gamalost intake[3]	−0.040	0.10	0.068	0.01	0.010	0.47	0.011	0.24	0.061	0.03
Sex, if male	0.106	0.57	−0.195	0.32	0.005	0.96	−0.244	0.001	0.102	0.62
Waist circumference	0.026	0.004	0.006	0.50	0.025	<0.001	−0.014	<0.001	0.017	0.08
Smoking, if yes	0.279	0.02	0.126	0.32	0.135	0.04	0.077	0.09	0.025	0.85

[1]Only significant factors are shown; other factors were analyzed but were not significant.

[2]B represents the mean change in blood pressure.

[3]Frequency of intake: 0, 0.5, 2.0, 5.0, 10.5 and 21.0 times/wk.

Diets aimed at reducing hypertension, such as the DASH diet (Dietary Approaches to Stop Hypertension; Sacks et al., 2001), often emphasize a high intake of low-fat dairy products. Gamalost contains only negligible amounts of fat (<1 g/100 g), making it suitable in these diets. The role of salt (NaCl) in the development of hypertension is much debated, but it is generally accepted that limiting salt intake is favorable in preventing hypertension. Salt is usually an important ingredient in the cheese-making process, contributing both to a desirable flavor and texture profile of the cheese and to microbial safety (Guinee and Fox, 2004). This, together with the high fat content of most cheeses, is the reason why cheese is generally not recommended in heart-healthy diets. Gamalost, however, is made without the addition of salt. Thus, if consumers eat Gamalost instead of other cheeses (rather than in addition to other cheeses), it could be argued that the concomitant reduction in fat and salt intake is the cause of the reduction in BP. However, as can be seen in Table 1, it seems that the most frequent consumers of Gamalost also have the highest intakes of all cheese and regular cheese. It is not possible from our study to assess if the found association between systolic BP and intake of Gamalost is due specifically to the high ACE-inhibitory potential of Gamalost, as the participants who consumed Gamalost most frequently also consumed more of other cheeses. Thus, they also consumed a higher amount of other nutrients found in cheese, such as calcium and potassium. Both dietary calcium and potassium have been shown to lower systolic and diastolic BP (Griffith et al., 1999; Houston, 2011) and to reduce the risk of hypertension in, for example, middle-aged and older women (Wang et al., 2008). In a study with hypertensive subjects, Calpis, a fermented milk that, like Gamalost, has a naturally high ACE-inhibitory potential, was compared with a placebo drink with equal calcium and potassium contents (Hata et al., 1996). In that study, a significant decrease in both systolic and diastolic BP was observed with Calpis only, suggesting that the effect was independent of the calcium and potassium contents.

The main strength of the present study is that a cheese shown to have a high ACE-inhibitory activity was asked about specifically in a food frequency questionnaire, not just as part of a question about total cheese or total dairy. The study sample was relatively homogeneous, in terms of ethnicity, place of birth, and environmental factors. Furthermore, all BP measurements were done under the same conditions—in the morning after an overnight fast. There was no interobserver error in the anthropometric measurements, because the same observer did all measurements. Even though the sample size was limited, we were able to recruit just under 10% of the eligible population in Vik i Sogn (total population of 2,768 in 2010). One limitation is the nature of the study design itself; namely, that it is a small cross-sectional study. The design cannot provide any evidence for a true cause-and-effect association, but the results may serve to suggest a direction for future research. To clarify whether intake of Gamalost does prevent hypertension and might reduce BP in hypertensive subjects, a large randomized intervention study would be needed. Therefore, an intervention trial comparing a possible BP-reducing influence of Gamalost with other types of cheese is currently in progress.

In summary, intake of Gamalost, a cheese with a high concentration of ACE-inhibiting peptides, was negatively associated with systolic BP in a Norwegian population with a high prevalence of Gamalost users. The association remained after controlling for sex, age, education, waist circumference, physical activity, smoking habits, and total intake of dairy products. Total dairy intake or total cheese intake was not associated with blood pressure in this study.

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PAPER II

Interpretive summary: Does a high intake of a cheese rich in ACE-inhibiting peptides lower blood pressure?

Nilsen

Cheese and some other dairy products contain bioactive compounds which may lower blood pressure in humans. High blood pressure is one of the biggest contributors to morbidity and mortality in the world. About 150 subjects participated in intervention this trial, investigating the effect of a high intake of two different cheeses on blood pressure. The results were compared against a control group who had a low cheese intake. We were unable to confirm that cheese could lower blood pressure at the amounts consumed in this trial.

RUNNING HEAD: EFFECT OF CHEESE INTERVENTION ON BLOOD PRESSURE

Effect of "Gamalost®", a cheese rich in angiotensin-converting enzyme (ACE)-inhibiting peptides, on blood pressure: results of a randomized trial.

Author list: **R. Nilsen[1]*, A. H. Pripp[†], A. T. Høstmark[‡], A. Haug[§], S. Skeie[*]**

[*]Department of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences, PO Box 5003, N-1432 Ås, Norway

[†]Oslo Centre of Biostatistics and Epidemiology, Research Support Services, Oslo University Hospital, N-0450 Oslo, Norway

[‡]Institute of Health and Society, University of Oslo, N-0450 Oslo, Norway

[§]Department of Animal and Aquacultural Sciences, Norwegian University of Life Sciences, N-1432 Ås, Norway

[1]Corresponding author:

Rita Nilsen

P.O. Box 5003, 1432 Ås, Norway

Telephone: (+47) 6496 5143 Fax: (+47) 6496 5001

E-mail address: rita.nilsen@nmbu.no.

ABSTRACT

High blood pressure (**BP**) is the leading risk factor for global disease burden, contributing to 7% of global disability adjusted life years. Angiotensin converting enzyme (**ACE**)-inhibiting bioactive peptides have the potential to reduce BP in humans. These peptides have been identified in many dairy products and have been associated with significant reductions in BP. The objective of this trial was to examine whether Gamalost®, a Norwegian cheese rich in ACE-inhibiting peptides, or a standard Norwegian Gouda-type cheese could lower BP. 153 healthy participants were randomized to one of three parallel arms: Gamalost® (*n* = 53, 50 g/day for 8 weeks), Gouda-type cheese (*n* = 50, 80 g/day for 8 weeks), and control (*n* = 50). Blood pressure and anthropometric measurements were taken at inclusion and end, with an additional BP measurement midway. Based on BP at inclusion, participants were categorised as having optimal BP (<120/<50 mmHg), normal-high BP (120-139/80-89 mmHg), or being hypertensive (>140/>90 mmHg). Questionnaires about lifestyle, health and dietary habits were completed at inclusion, midway and end. In total 148 participants (mean age 43, 52% female) completed the intervention. At baseline, there were no differences between the three groups. Blood pressure was reduced in the entire study population, but the cheese groups did not differ from control. However, in a subgroup of participants with slightly elevated BP, BP at four weeks of intervention seemed to be borderline significantly more reduced in the Gamalost® group compared with the control group (Dunnett test: diastolic BP -3.5 mmHg, 95% confidence interval (**CI**) -7.3, 0.4, systolic BP: -4.3 mmHg, 95% CI -9.8, 1.1). Intention-to-treat analysis of the data showed no cheese effect upon BP compared to control, but Gamalost® seemed to have a small non-significant lowering effect on diastolic BP after four weeks in people with a normal-high BP.

Key words: “blood pressure”, cheese, “ACE-inhibiting peptide”, “human trial”

INTRODUCTION

Cardiovascular diseases (**CVD**) are the most common contributors to worldwide morbidity and mortality (Alwan, 2011), and ischemic heart disease is the leading cause of death in the world (WHO, 2014). Hypertension is a major risk factor for CVD, and it has been estimated from prospective observational studies that just a 5 mmHg reduction in diastolic blood pressure (**BP**) would reduce the risk of stroke by 34% (MacMahon *et al*, 1990). Blood pressure was identified as the leading risk factor contributing to global disease burden in "the Global Burden of Disease Study 2010", and it was estimated that 16.5% of all deaths can be attributed to high BP (Lim *et al*, 2012).

Hypertension is mostly treated pharmacologically, but lifestyle and dietary changes such as weight loss and reduced salt intake have been effective in preventing hypertension (Appel *et al*, 2006). The Dietary Approaches to Stop Hypertension (**DASH** diet), which emphasizes a high intake of dairy and fruits and vegetables, is one of the trials showing that diet is a successful tool used to reduce hypertension (Sacks *et al*, 2001). Dairy products are rich sources of protein, calcium and potassium which have all been shown to independently reduce BP (He *et al*, 2001; Wang *et al*, 2008; Houston, 2011). Dairy proteins are also one of the main sources of bioactive peptides in the human diet (Korhonen and Pihlanto, 2006), which are present in varying amounts in different cheeses. These bioactive peptides have several known activities, including angiotensin-converting enzyme (**ACE**) inhibition. The function of ACE is to activate angiotensin II, a vasoconstrictor, as well as inactivating bradykinin, a vasodilator (Silva and Malcata, 2005), resulting in increased BP. ACE-inhibiting peptides have been identified in several cheeses and other fermented milk products, including Cheddar (Pritchard *et al*, 2010), Manchego (Gomez-Ruiz *et al*, 2002), Asiago (Lignitto *et al*, 2010) and the traditional

Norwegian cheese Gamalost® (Qureshi *et al*, 2012). Some randomized controlled trials have shown that fermented milks and extracts of ACE-inhibiting peptides from milk products can reduce BP in humans, and a meta-analysis showed that food derived peptides, such as the two lactotripeptdies valine-proline-proline (**VPP**) and isoleucine-proline-proline (**IPP**), had the potential to lower systolic BP by 5 mmHg, by the pooled effect of amounts ranging from 1.5 mg VPP and 1.1 mg IPP, to 30 mg VPP and 22.5 mg IPP (Pripp, 2008).

Gamalost® is a cheese made from skimmed milk that does not contain salt and is naturally low in fat (<1%) and very rich in protein (50%). A detailed account of the production of Gamalost® has been described previously (Qureshi *et al,* 2012). The cheese is rich in bioactive peptides and was found to have a better ACE-inhibitory activity, in terms of concentration of cheese peptides needed to inhibit 50% of ACE, than other cheeses (Qureshi *et al,* 2012; Pripp *et al,* 2006; Qureshi *et al,* 2013). Compared to cheeses from other studies Gamalost was one of the cheeses with the highest ACE-inhibitory potential (Sieber *et al*, 2010). A cross-sectional study on Gamalost® and BP was carried out in 2012 and showed that Gamalost® intake frequency was associated with slightly lower systolic BP (Nilsen *et al*, 2014). We are not aware of any previously published randomized controlled trials specifically investigating the effect of cheese on BP.

The aim of this work was to investigate whether consumption of cheese might lower blood pressure during eight weeks of intervention.

MATERIALS AND METHODS

Subjects

Participants were recruited through local newspapers, radio and television, from the general population. The target population was persons with moderately high blood BP, who were not medicated. Males and females over 18 years of age who spoke Norwegian fluently were included. Exclusion criteria included pregnancy and use of BP lowering medications.

Design

The study performed was a randomized single-blinded controlled trial with three parallel arms, as illustrated in figure 1. The intervention period lasted for eight weeks with measurements taken at baseline, midway and at the end of the trial. An independent person not involved in the study prepared the randomization envelopes containing information on which intervention the participants would follow. Independent of baseline BP, the participants were handed envelopes by two independent persons not involved in the conduct of the study. The sample size estimate for one-way analysis of variance with three groups, with a power of 0.80 and criterion for significance set at 0.05, yielded a sample size of 53 cases per group and a total of 159. We initially aimed for a larger sample of about 300 participants, but recruitment yielded a total sample size of 153.

This study was carried out at the Department of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences, Aas, Norway from April 2013 to July 2013 and was approved by the Regional Committees for Medical and Health Research Ethics (Oslo, Norway) on 7th March, 2013 (2013/166) (registered at www.clinicaltrials.gov; NCT01913756). The study was conducted according to the guidelines laid down in the Declaration of Helsinki and written informed consent was obtained from all subjects.

126

127 ***Interventions***

128 Participants were randomized to one of three arms: Gamalost®, Gouda-type cheese, or control.
129 Whereas the bitter taste and crumbly texture prevents Gamalost from being widely consumed,
130 mild and versatile Gouda-type cheeses have the highest consumption in Norway. A simulated
131 human gastrointestinal digestion trial showed that the moderate ACE-inhibitory activity of
132 Gouda-type cheeses increased greatly after digestion (Qureshi *et al*, 2013), making the cheese
133 an interesting addition to this trial. All participants in the cheese groups were asked to maintain
134 their habitual diet and not make any other major lifestyle changes. Subjects in the control group
135 were asked to maintain their habitual diet, but to avoid the two intervention cheeses as well as
136 similar cheeses. They were given lists of suggestions for other cheeses they could freely
137 consume, such as blue cheese, mozzarella cheese or cream cheese.

138

139 Participants in the Gamalost® group were instructed to consume 50 g/day of the cheese, whereas
140 participants in the Gouda-type cheese group were instructed to consume 80 g/day of the cheese.
141 These amounts were chosen because they were judged to be higher than the average intake of
142 each cheese, but not so high that the participants were unable to consume the designated
143 amount. In order to have similar cheese protein intakes in the two cheese groups, the Gouda-
144 type intervention was larger than the Gamalost® intervention. The cheeses were not portioned,
145 but the participants were provided with digital scales to accurately weigh out the daily intake.
146 All the Gamalost® cheeses were from the same batch and were ripened for 10 days. All the
147 Gouda-type cheeses were from the same batch and were ripened for three months. Furthermore,
148 participants were instructed to freeze the Gamalost® cheese throughout the trial so that the rapid
149 proteolysis occurring in the cheese would not change the activity of the ACE-inhibiting

peptides, as freezing does not affect the ACE-inhibitory activity of the cheese. The nutritional properties and ACE-inhibitory activity of the two cheeses can be found in table 1.

Socio-demographic and Dietary Assessment

A questionnaire was developed for a cross-sectional trial on Gamalost® intake and BP in a Norwegian population (20), and it was based on the previously validated questionnaire from the Oslo Health Study (main questionnaire and second supplementary questionnaire 1 of the Oslo Health Study). Experience from this cross-sectional trial showed that the questionnaire was suitable, but a couple of more questions on food intake were added. The questionnaire contained questions on age, education, health, leisure time physical activity, medication and supplement use, as well as several questions on diet, including some focusing specifically on dairy product intake. A translated version of the questionnaire used at inclusion can be found in appendix 1. Total dairy product intake was calculated by summarizing the frequency of intake of all cheese, all milk, and fermented milk products. A revised version of the questionnaire containing only questions on food intake was distributed at the midway measurements, whereas a third version, which included some of the questions on health and physical activity from the first questionnaire, was used at the end of the trial. The second and third questionnaires were used to assess whether any major changes to diet and physical activity pattern occurred during the intervention period. Participants were also asked to record any difficulties they had with compliance. The last questionnaire also included a question regarding discomforts the participants may have experienced during the intervention period.

Blood Pressure Measurements

Blood pressure was measured according to the American Heart Association recommendations (Pickering *et al,* 2005). Participants rested for approximately 10 minutes before the

measurement was taken using a Microlife BP A200 sphygmomanometer (Microlife, Widnau, Switzerland). In a sitting position, three consecutive measurements were taken and the average of the second and third measurements was used for analysis (automatically calculated by the BP device). Where needed, the device took four measurements to get a more accurate reading. All BP measurements were taken between 06:30 and 10:30, after an overnight fast. Participants were notified of their BP and whether the BP was within the normal range or not. Based on baseline BP, participants were grouped into categories according to the guidelines published by the European Society of Hypertension and the European Society of Cardiology (Mancia *et al,* 2014). Consequently, participants were categorized as optimal if systolic BP was <120 mmHg and diastolic BP was <80 mmHg, normal-high if systolic BP was 120-139 mmHg and/or diastolic BP was 80-89 mmHg, and hypertensive if systolic BP was ≥140 mmHg and/or diastolic BP was ≥90 mmHg.

Anthropometric Measurements

Height was measured to the nearest 0.1 cm using a portable stadiometer (Seca 217, Seca, Hamburg, Germany). Body weight was measured to the nearest 0.1 kg, without shoes or heavy clothing, using digital scales (TBF-300A Body Composition Analyzer, Tanita, Tokyo, Japan). Body mass index (**BMI**) was computed as weight (kg) divided by the square of height (m). Waist circumference was measured to the nearest 0.1 cm using a measuring tape (Seca 201 Circumference measuring tape, Seca), in accordance with World Health Organization recommendations, i.e. at the midpoint between the iliac crest and the lowest rib margin (WHO, 2011). All anthropometric measurements were also performed between 06:30 and 10:30.

Statistical Analyses

Prior to statistical analysis, the dataset was recoded by an independent person to remove information on intervention groups, hence the primary researchers were blinded while performing the analyses. Statistical analyses were performed according to the intention to treat principle. One-way ANOVA with Bonferroni correction for multiple comparisons or the chi-square (χ^2) test were used as appropriate to assess differences between the intervention groups. Baseline characteristics are presented as mean (standard deviation) or percentages where appropriate. The paired samples t-test was used to evaluate change in BP from start to end in each intervention group. Dunnett test was used to evaluate mean BP changes between each treatment group and the control group. A *P*-value <0.05 was considered statistically significant. All statistical analyses were performed using the SPSS 21.0 software package (IBM Corporation, Armonk, New York).

RESULTS

Baseline Characteristics

A total of 153 participants were included in the study from the beginning (n = 53 in Gamalost® group, n = 50 in both Gouda-type cheese and control groups). At baseline 22% had optimal BP, 46% had moderately high BP, and 31% were hypertensive. Some baseline characteristics of the total study sample and the three groups are presented in table 2. There were no major differences between the groups in selected health variables, salt, alcohol or dairy product intake. Blood pressure was not significantly different at baseline, even though the prevalence of hypertension was slightly lower in the Gouda-type cheese group compared to the control group. The participants consumed on average seven servings of cheese per week, whereas Gamalost® intake was expectedly low with less than one serving per week.

At the end of the intervention waist circumference decreased significantly in all three intervention groups (P <0.001), whereas weight was only significantly reduced in the Gamalost® and control groups (data not shown). Dairy product and Gamalost® intake was significantly different between the groups at the end of the trial (ANOVA, data not shown, P <0.001), with total dairy intake increased in the two cheese groups and decreased in the control group. Five participants failed to complete the trial, as illustrated in figure 1, due to adverse events unrelated to the study itself.

Blood Pressure Changes

Paired samples t-test (table 3) showed that both systolic and diastolic BP decreased significantly from baseline to midway, and baseline to end of trial in the entire study population. All groups had significant decreases in systolic BP (figure 2A, table 3), whereas at the end of the trial only the Gamalost® group had significantly decreased diastolic BP (figure 2B, table 3). Intention-to-

235 treat analysis of BP change, and comparing the intervention groups with the control group,
236 showed no effect of the cheeses on midway or end BP (table 4).

237 When stratifying by baseline BP category, the paired samples t-test (appendix 2) showed that
238 the participants with optimal BP at baseline did not have any reductions throughout the trial, as
239 illustrated in figure 3. Figure 3 A and B shows mean systolic and diastolic BP changes through
240 the trial, respectively, grouped by BP category at inclusion. Systolic BP decreased significantly
241 in the hypertensive subgroup in both the Gamalost® (P = 0.001) and control (P <0.001) groups
242 at both midway and end measurements. Systolic BP was also significantly lower at the end of
243 the trial (P = 0.049) for participants with normal-high BP in the Gouda-type cheese group. At
244 the end of the trial, diastolic BP was significantly decreased in the normal-high BP (P = 0.038)
245 and hypertensive (P = 0.004) subgroups of the Gamalost® intervention group only.

246 Further analyses stratified by BP categories are presented in table 5. Here, the intervention
247 cheese groups are compared against the control group. Systolic BP shows a borderline
248 significant effect of consuming Gamalost® compared to control also in the normal-high BP
249 subgroup (-4.3 mmHg, 95% CI -9.8, 1.1, P = 0.14). Even though BP decreased overall in all
250 groups, when comparing change in BP in those with hypertension at baseline, the Gouda-type
251 cheese group had significantly higher midway and end systolic BP compared to the control
252 group (midway: 10.5 mmHg, 95% CI 0.9, 20.2, P = 0.03, end: 10.1 mmHg, 95% CI 1.6, 18.6,
253 P = 0.02). In the Gamalost® group, there seems to be a small borderline significant effect on
254 diastolic BP compared to control at week 4, for participants with normal-high BP at baseline (-
255 3.5 mmHg, 95% CI -7.3, 0.4, P = 0.08). At 8 weeks, the association remained but less
256 significant.
257

DISCUSSION

This randomised controlled trial showed systolic BP reductions in all intervention groups, and reductions in diastolic BP in the Gamalost® group after eight weeks of intervention. However, compared to the control, there was no BP lowering effect of Gamalost®, a cheese rich in ACE-inhibiting peptides, or of a standard Gouda-type cheese in 153 subjects recruited from a general healthy population. When participants were stratified by baseline BP, there was a non-significant effect of consuming Gamalost® compared to control at four weeks in participants with normal-high BP, but this was not present at eight weeks.

In this trial, systolic BP was significantly reduced in hypertensive subjects in both the Gamalost® and control groups, but not the Gouda-type group. It is uncertain why BP was reduced in the control group, but it could be a result of the statistical phenomenon known as regression to the mean (Barnett *et al*, 2005). Even though there was no significant difference in baseline BP between the three groups, the Gouda-type cheese group had fewer subjects with hypertension compared to the Gamalost® and control groups. This could possibly explain why the hypertensive subgroup in the Gouda-type cheese group did not have the same reductions in BP as the other two groups. It was hypothesised that during the intervention, BP would decrease in the Gamalost® group compared to the control, due to the high intake of ACE-inhibiting bioactive peptides. A borderline significant reduction in diastolic BP in the Gamalost® group was seen in the subcategory of subjects with normal-high BP. The association was less significant at eight weeks, which could be explained by regression to the mean or problems with compliance. This borderline significant change in diastolic BP is in accordance with a larger diet intervention trial on the effect of the Mediterranean diet on BP, which found a small significant effect on diastolic but not systolic BP (Toledo *et al*, 2013). In the previous cross-sectional trial (Nilsen *et al*, 2014), the opposite occurred as systolic BP, but not diastolic BP,

was significantly lower with higher intakes of Gamalost®. With the high prevalence of high BP and hypertension in the Norwegian and worldwide population, a small lowering of mean BP in the normal-high BP subgroup in the Gamalost® group compared to control at four weeks could be clinically meaningful if they were able to achieve a significant effect beyond four weeks. The normal-high BP category was the largest subgroup in all three intervention groups, leading to the assumption that results from these groups have the best statistical power. In the general population, this subgroup of people is also those who might benefit from lifestyle changes such as a diet including a cheese rich in ACE-inhibiting peptides, such as Gamalost®.

It has been estimated that a small reduction in diastolic BP of 2 mmHg could reduce the risk of coronary heart disease by 6% (Cook *et al,* 1995), indicating that only small reductions are needed for cheese and dairy products to have a clinically meaningful effect on BP. Previous randomized controlled trials on the BP lowering effect on milk-derived bioactive peptides showed mixed results. VPP and IPP, derived from casein, are usually considered the two lactotripeptides with the most promising antihypertensive potential (Engberink *et al,* 2008). However, in a double-blinded placebo-controlled trial on subjects with elevated BP (SBP ≥140 mmHg) given concentrates of these two peptides, they did not exert any BP lowering effect compared to the placebo group (Engberink *et al,* 2008). A similar trial using a milk fermented with *Lactobacillus helveticus,* which gave a product naturally rich in VPP and IPP, found a significant BP lowering effect on diastolic BP which was not maintained after four weeks intervention in the normal-high BP category, whereas both systolic and diastolic BP was decreased in the hypertensive category until four weeks (Aihara *et al,* 2005). Many trials investigating the BP lowering effect of foods have used extracts of foods or synthetic foods/drinks containing active ingredients which may occur naturally in foods. Trials that use actual foods as the intervention, such as the current trial, have mixed results in terms of BP

reductions. A BP lowering effect of foods consumed as part of a normal diet in a free living population has been observed for foods such as kiwifruit (male smokers) (Karlsen *et al*, 2013), flaxseed (peripheral artery disease patients) (Rodriguez-Leyva *et al*, 2013) and fermented milk (buttermilk) (moderately hypercholesterolemic subjects) (Conway *et al*, 2014), whereas no significant effects were found for other foods such as walnuts (Katz *et al*, 2012).

Information obtained from participants in the cross-sectional trial (Nilsen *et al*, 2014) indicated that 50 grams of Gamalost® was a feasible daily intake for people who are regular consumers of Gamalost®. It is possible that a true BP lowering effect of Gamalost® would be observed if the serving was increased, but we judged that a bigger serving would be unlikely to be tolerated by regular consumers. The study population in the current intervention trial was recruited from the general population and they had no underlying conditions and diseases. We were unable to recruit solely subjects with increased BP at baseline, as evidenced by the initial BP in the overall study population (132/82 mmHg). A significant correlation between initial BP and change in BP has been found (Summer *et al*, 1988), with initial higher BP showing greater response to the BP lowering agent, indicating that should further studies on the BP lowering effect of Gamalost® be performed, it is suggested that subjects with optimal BP be excluded from the trial. Even though many cheeses have been found to be rich in ACE-inhibiting bioactive peptides, the results from the present trial suggest that the effect may not be transferrable to healthy human populations at the amount of cheese consumed in this trial.

The main limitation of this trial is the use of a sphygmomanometer for in office BP measurements as opposed to ambulatory 24 hour BP measurements, which would produce values of higher accuracy. Blood pressure varies throughout the day and the current trial is therefore unable to distinguish whether the participants had an effect of the intervention on

nocturnal BP. White-coat hypertension, reported to occur in about 15-35% of people (Pickering *et al*, 2005; Franklin *et al*, 2013; Pickering *et al*, 1999), is a source of error which could be greatly reduced by ambulatory BP measurements (O'Brien *et al*, 2013). If the baseline BP measurements were falsely high, it is expected that BP will decrease slightly on subsequent visits, a result of getting used to the situation and regression to the mean. However, subjects were randomly allocated to groups and statistical analyses were adjusted according to the subject's baseline BP. At the baseline BP measurement, the participants were informed of their BP. They were not given any medical advice, but they were told if the BP was outside of the recommended range that they could make an appointment with their general practitioner. None of the participants reported starting any medical antihypertensive treatments during the trial. Furthermore, since the participants were of generally good health and had a normal BP at baseline, the generalizability of these findings to populations with a higher BP may be somewhat limited. The participants were only provided with the intervention cheese, and while they were asked to maintain their habitual diet, we had only partial control of the diet during the trial.

The strengths of this study include the relatively long duration and the design of the trial itself, specifically that it is a randomized single-blinded controlled trial. Retention of participants in the trial was good, with only five subjects lost to follow-up, and the same number of subjects dropped out in the Gamalost® group and the control group.

In conclusion, when comparing to the mean change in BP in the control group, there was no major effect of a cheese rich in ACE-inhibiting peptides or a standard Gouda-type cheese on BP in a general population. However, when stratified by BP category at inclusion, there was a non-significant reduction in diastolic BP in the Gamalost® group compared to control in participants with normal-high BP at four weeks of intervention. The current results suggest

cheeses rich in ACE-inhibiting bioactive peptides may not have an effect on BP when consumed in moderate amounts, but further similar trials on other cheeses should be performed to evaluate these findings.

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The authors' contributions are as follows: all authors contributed to formulating the research question, designing the study, carrying out the statistical analysis and critically revising the manuscript. Additionally, R. N. carried out the study and drafted the manuscript.

None of the authors have any conflicts of interest to declare.

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Tables

Table 1. Nutrient composition (per 100 g and daily intake) of intervention cheeses

Nutrient	Gamalost®		Gouda-type cheese	
	100 g	Daily intake, 50 g	100 g	Daily intake, 80 g
Energy[1], kcal	213	107	351	281
Protein[1], g	50	25	27	22
Fat[1], g	1	0.5	27	22
Carbohydrates[1], g	1	0.5	0	0
Calcium[1], mg	160	80	800	640
Sodium[1], mg	24	12	402	322
Magnesium[1], mg	13	7	33	26
Potassium[1], mg	98	49	77	62
IC_{50} ACE-inhibition[2]	0.34	0.34	0.59	0.59
ACE-inhibitory potential[3], mg	0.24	0.12	0.03	0.02

[1]From TINE SA, manufacturer of the two cheeses

[2]IC_{50} per unit weight of freeze-dried pH 4.6 soluble fraction (**SF**), expressed as mg pH 4.6 SF per ml. From Qureshi *et al*, 2012

[3]ACE-inhibitory potential, expressed as mg captopril equivalents per cheese weight. From Qureshi *et al*, 2012

Table 2. Baseline characteristics (mean (SD[1])) or %, by intervention group

	Intervention group								
	All (*n*=153)		Gamalost® (*n*=53)		Gouda-type cheese (*n*=50)		Control (*n*=50)		
Characteristic	Mean	SD	Mean	SD	Mean	SD	Mean	SD	*P*
Gender, female (%)	52.3		50.9		60.0		46.0		0.4
Age (years)	43.1	16.4	41.2	17.0	42.7	15.8	45.5	16.4	0.4
Weight (kg)	77.2	14.8	75.6	13.7	76.0	13.6	79.9	16.8	0.3
Height (cm)	173.9	8.9	174.9	8.7	171.9	8.7	174.7	9.4	0.2
BMI (kg/m^2)	25.7	3.7	24.6	3.3	25.6	3.5	26.0	3.7	0.1
Waist circumference (cm)	83.1	11.8	80.9	11.3	82.8	10.9	85.8	12.9	0.1
Systolic BP (mmHg)	132.3	17.2	131.5	19.3	130.6	14.7	134.8	17.2	0.4
Diastolic BP (mmHg)	82.4	9.8	82.5	10.6	81.4	8.9	83.1	10.0	0.7
Hypertension[2] (%)	31.4		34.0		24.0		36.0		0.4
Education (years)	16.6	2.9	16.6	2.7	17.0	2.3	16.4	3.5	0.6
Smoking[3] (%)	3.3		3.8		2.0		4.1		0.9
Physical activity[4] (%)	38.4		39.6		36.7		38.8		0.9
Salt usage[5] (%)	72.5		71.7		72.0		74.0		0.9
Alcohol consumption[6] (%)	57.2		64.2		53.1		54.0		0.1
Total dairy[7]	18.4	11.9	19.7	12.9	17.5	10.1	18.0	12.7	0.6
Gouda-type cheese[7]	5.7	4.3	6.1	4.6	5.4	3.6	5.6	4.8	0.7
Gamalost®[7]	0.7	1.9	0.6	1.7	0.6	1.8	0.8	2.2	0.9

[1]SD, standard deviation
[2]Percentage who have either SBP>140, or DBP>90
[3]Percentage daily smokers
[4]Percentage who reported moderate to hard physical activity more than four hours per week
[5]Percentage who salt their food
[6]Percentage who consume alcohol >1/week
[7]Servings per week

Table 3. Mean difference (MD[1]) with 95% CI in blood pressure in each group, paired samples t-test comparing start to 4 and 8 weeks follow-up.

		Study population			Gamalost®			Gouda-type cheese			Control		
		MD	95% CI	*P*	MD	95% CI	*P*	MD	95% CI	*P*	MD	95% CI	*P*
Systolic BP[2], mmHg	Change to 4 weeks	-2.7	-4.2, -1.1	0.001	-3.9	-6.5, -1.3	0.004	-0.5	-3.2, 2.3	0.727	-3.5	-6.5, -0.5	0.023
	Change to 8 weeks	-4.4	-6.0, -2.9	<0.001	-4.4	-7.0, -1.8	0.001	-3.5	-5.8, -1.3	0.003	-5.4	-8.5, -2.2	0.001
Diastolic BP, mmHg	Change to 4 weeks	-2.3	-3.3, -1.3	<0.001	-3.8	-5.3, -2.2	<0.001	-0.7	-2.4, 1.0	0.421	-2.3	-4.4, -0.3	0.029
	Change to 8 weeks	-1.6	-2.7, -0.6	0.002	-2.9	-4.4, -1.4	<0.001	-0.9	-2.7, 0.9	0.319	-1.1	-3.3, 1.1	0.333

[1]MD, mean difference

[2]BP, blood pressure

Table 4. Blood pressure changes during intervention, comparing control group with the two cheese diets. Values are mean (SE), 2-sided *P*-values for the difference from control (Dunnett test)

		4 weeks (midway)				8 weeks (end)			
	Baseline BP	Mean BP	BP change	Difference from control (95% CI)	*P*	Mean BP	BP change	Difference from control (95% CI)	*P*
Systolic BP[1]									
Control	134.8 (2.4)	131.8 (2.1)	-3.5 (1.5)			129.8 (2.0)	-5.4 (1.6)		
Gamalost®	131.5 (2.7)	128.0 (2.6)	-3.9 (1.3)	-0.4 (-4.8, 3.9)	0.97	128.0 (2.2)	-4.4 (1.3)	1.0 (-3.2, 5.2)	0.83
Gouda-type	130.6 (2.1)	130.5 (2.4)	-0.5 (1.4)	3.0 (-1.4, 7.4)	0.23	127.4 (2.4)	-3.5 (1.1)	1.8 (-2.4, 6.1)	0.53
Diastolic BP									
Control	83.1 (1.4)	80.9 (1.5)	-2.3 (1.0)			82.1 (1.4)	-1.1 (1.1)		
Gamalost®	82.5 (1.5)	79.1 (1.5)	-3.8 (0.8)	-1.5 (-4.2, 1.3)	0.40	80.2 (1.4)	-2.9 (0.7)	-1.8 (-4.6, 1.1)	0.28
Gouda-type	81.4 (1.3)	80.9 (1.4)	-0.7 (0.8)	1.6 (-1.2, 4.5)	0.33	80.7 (1.4)	-0.9 (0.9)	0.2 (-2.7, 3.1)	0.98

[1]BP, blood pressure

Table 5. Stratified analysis based on blood pressure category at baseline, comparing control group with the two cheese diets. Values are mean (SE), 2-sided *P*-values for the difference from control (Dunnett test)

			4 weeks (midway)				8 weeks (end)			
		Baseline BP	Mean BP	BP change	Difference from control (95% CI)	*P*	Mean BP	BP change	Difference from control (95% CI)	*P*
Systolic BP[1]										
Hypertensive	Control	152.7 (3.1)	141.8 (3.3)	-10.9 (2.2)			139.1 (2.9)	-13.7 (2.5)		
	Gamalost®	150.8 (4.4)	144.2 (4.6)	-6.7 (2.8)	4.3 (-4.3, 12.9)	0.43	141.3 (3.9)	-9.50 (2.5)	4.2 (-3.4, 11.8)	0.36
	Gouda-type	151.5 (2.6)	147.9 (3.5)	-0.4 (3.7)	10.5 (0.9, 20.2)	0.03	147.9 (3.5)	-3.6 (2.1)	10.1 (1.6, 18.6)	0.02
Normal-high	Control	129.2 (2.1)	129.7 (2.1)	0.3 (1.7)			128.7 (1.9)	-0.6 (1.7)		
	Gamalost®	128.4 (1.4)	124.4 (2.2)	-4.1 (1.7)	-4.3 (-9.8, 1.1)	0.14	125.4 (1.9)	-3.0 (1.7)	-2.4 (-8.1, 3.4)	0.55
	Gouda-type	128.6 (1.0)	127.9 (1.6)	-0.5 (1.5)	-0.8 (-6.0, 4.3)	0.91	124.9 (2.1)	-3.5 (1.7)	-2.9 (-8.3, 2.5)	0.37
Optimal	Control	111.5 (1.4)	114.9 (2.8)	3.4 (2.4)			111.8 (2.6)	0.3 (2.2)		
	Gamalost®	112.5 (1.3)	112.3 (2.5)	-0.1 (1.9)	-3.5 (-11.7, 4.7)	0.50	113.5 (2.0)	0.5 (1.6)	0.2 (-6.8, 6.4)	1.0
	Gouda-type	112.9 (1.3)	127.9 (1.6)	-0.4 (3.1)	-3.8 (-12.5, 5.0)	0.50	109.7 (2.3)	-3.5 (1.9)	-3.8 (-10.2, 2.7)	0.31
Diastolic BP										
Hypertensive	Control	91.9 (2.2)	141.8 (3.3)	-5.1 (2.0)			88.5 (2.3)	-3.4 (2.1)		
	Gamalost®	92.2 (2.6)	144.2 (4.6)	-4.1 (1.4)	1.0 (-4.5, 6.5)	0.89	87.6 (2.6)	-4.6 (1.4)	-1.2 (-6.8, 4.4)	0.85
	Gouda-type	92.0 (2.3)	147.9 (3.5)	-0.9 (2.1)	4.1 (-2.0, 10.3)	0.23	92.3 (2.5)	-0.8 (1.8)	2.6 (-3.6, 8.9)	0.54
Normal-high	Control	80.8 (1.0)	80.5 (1.6)	-0.1 (1.3)			80.6 (1.5)	0.1 (1.5)		
	Gamalost®	81.0 (1.2)	77.5 (1.1)	-3.6 (1.1)	-3.5 (-7.3, 0.4)	0.08	78.8 (1.3)	-2.2 (1.0)	-2.2 (-6.4, 2.1)	0.42
	Gouda-type	80.9 (0.8)	79.3 (1.1)	-1.4 (1.0)	-1.3 (-4.9, 2.4)	0.65	79.7 (1.3)	-0.9 (1.3)	-0.9 (-4.9, 3.1)	0.84
Optimal	Control	70.5 (1.2)	68.5 (1.2)	-2.0 (1.3)			71.8 (2.4)	1.3 (1.8)		
	Gamalost®	73.0 (1.0)	69.7 (1.9)	-3.7 (1.7)	-1.7 (-7.7, 4.3)	0.72	71.9 (1.4)	-1.5 (1.5)	-2.8 (-8.0, 2.4)	0.36
	Gouda-type	71.3 (1.5)	79.3 (1.1)	1.3 (2.0)	3.3 (-3.1, 9.7)	0.38	71.2 (1.1)	-0.8 (1.3)	-2.1 (-7.5, 3.4)	0.59

[1]BP, blood pressure

Figure legends

Figure 1. Flow chart of a single-blinded, randomized, controlled trial of Gamalost® and Gouda-type cheeses and blood pressure in 153 Norwegian participants. BP, blood pressure.

Figure 2. Mean values (standard deviation) for systolic (A) and diastolic (B) blood pressure at inclusion, midway and end in the three groups. ○Gamalost®, ●Gouda-type cheese, ▵Control

Figure 3. Systolic (A) and diastolic (B) blood pressure (mean (SD)) at inclusion, midway and end, in three intervention groups. Solid lines: participants with hypertension at inclusion; dashed lines: participants with normal-high BP at inclusion, and dotted lines: participants with optimal BP at inclusion. ○Gamalost®, ●Gouda-type cheese, ▵Control

Figure 1.

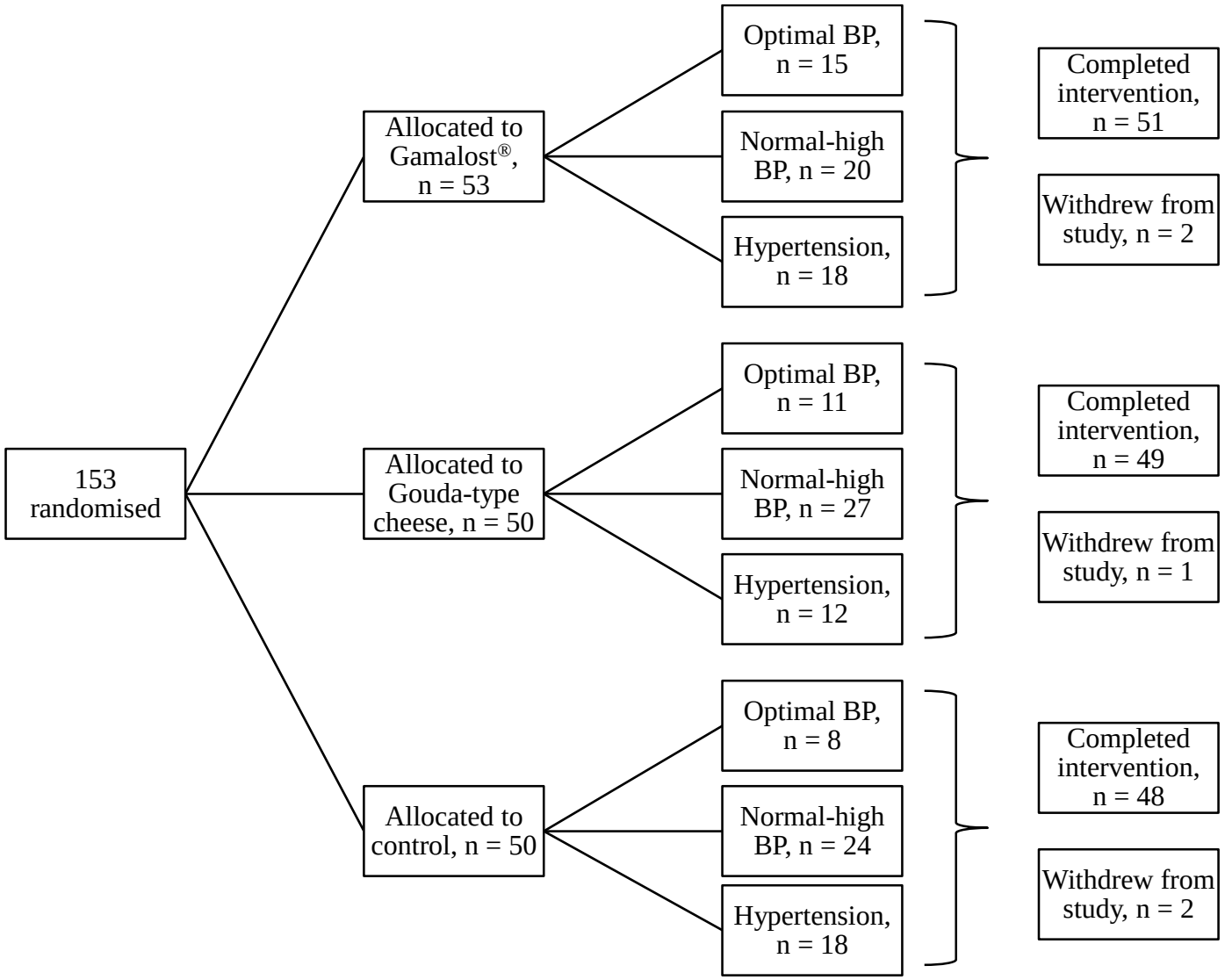

153 randomised
Allocated to Gamalost®, n = 53
Allocated to Gouda-type cheese, n = 50
Allocated to control, n = 50
Optimal BP, n = 15
Normal-high BP, n = 20
Hypertension, n = 18
Optimal BP, n = 11
Normal-high BP, n = 27
Hypertension, n = 12
Optimal BP, n = 8
Normal-high BP, n = 24
Hypertension, n = 18
Completed intervention, n = 51
Withdrew from study, n = 2
Completed intervention, n = 49
Withdrew from study, n = 1
Completed intervention, n = 48
Withdrew from study, n = 2

Figure 2.

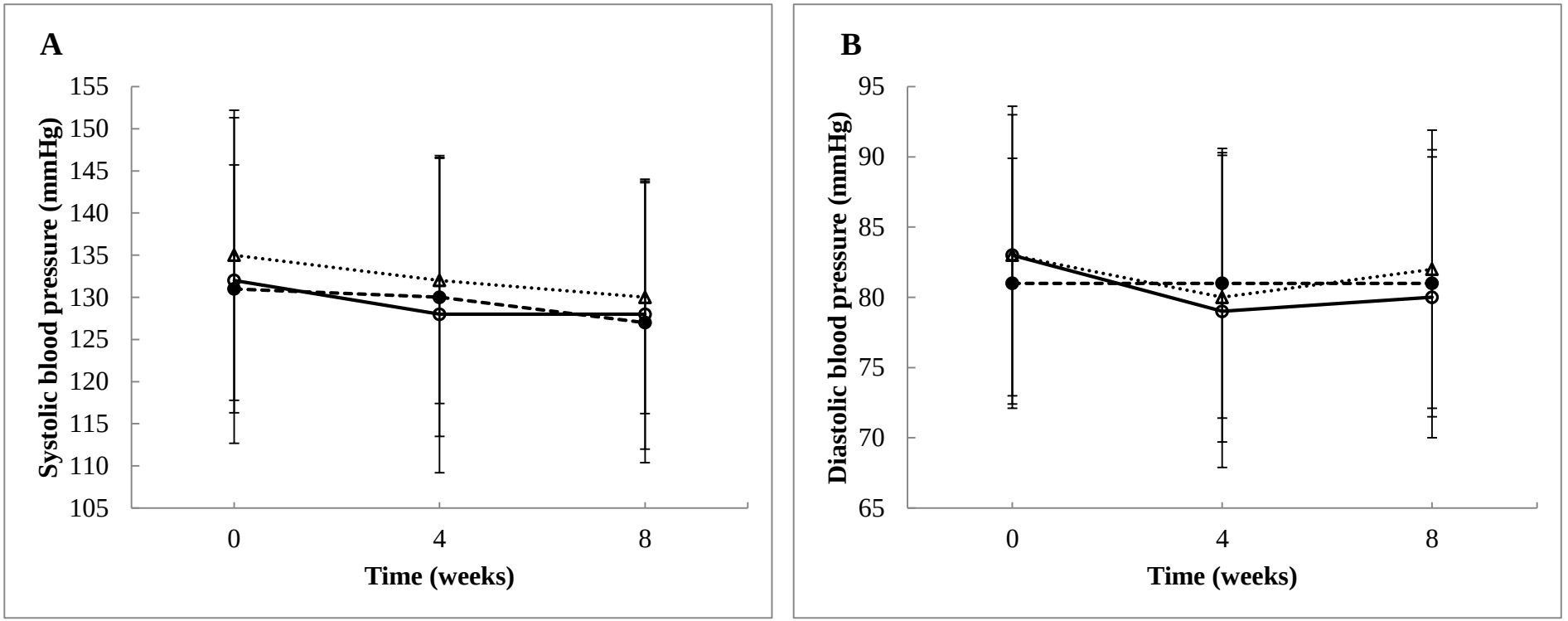
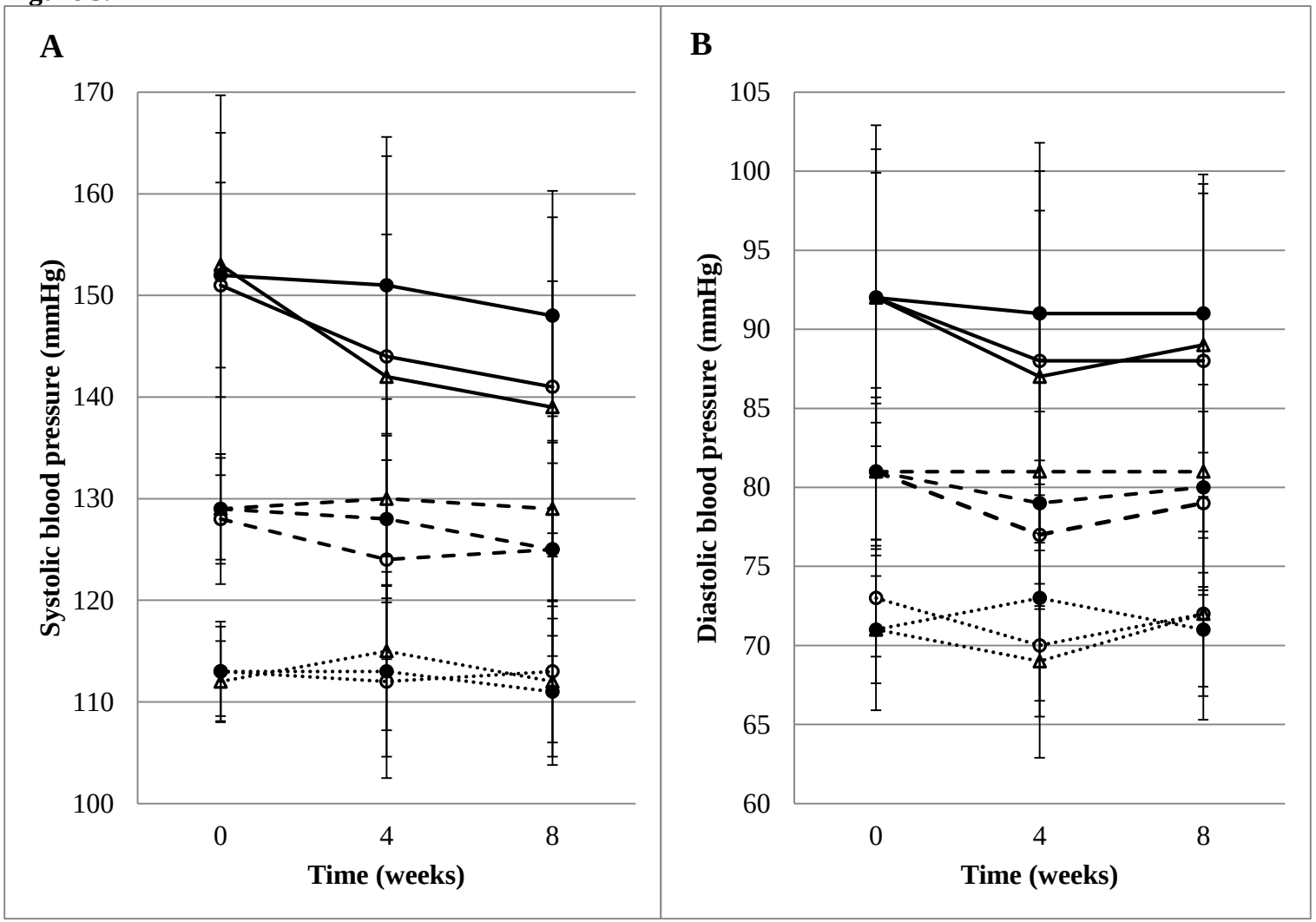

A
Systolic blood pressure (mmHg)
155
150
145
140
135
130
125
120
115
110
105
0
4
8
Time (weeks)
B
Diastolic blood pressure (mmHg)
95
90
85
80
75
70
65
0
4
8
Time (weeks)

Figure 3.


A
170
160
150
140
130
120
110
100
Systolic blood pressure (mmHg)
0
4
8
Time (weeks)
B
105
100
95
90
85
80
75
70
65
60
Diastolic blood pressure (mmHg)
0
4
8
Time (weeks)

Appendix

Appendix 1. Questionnaire at inclusion

1. Your health

1.1 How would you describe your present state of health? (Check only one answer)

Poor	Not very good	Good	Very good

1.2 Do you have any of these illnesses, or have you suffered from of them in the past?

	Yes	No	Age on first occasion
Asthma			
Chronic bronchitis/emphysema			
Diabetes			
Osteoporosis			
Myocardial infarction			
Angina pectoris (cardiac spasm)			
Stroke/cerebral haemorrhage (“drip”)			
High blood pressure			

2. Where you grew up/where you live

2.1 Where did you live for most of the time before you reached the age of 16 years? (Check one alternative and specify)

Same place	
Another county in Norway	County:
Outside Norway	Country:

2.2 Have you moved in the course of the last five years? (Check only one answer)

No	Yes, once	Yes, several times

3. Weight

3.1 Assess your weight when you were 25 years | | kg | old:

4. Food and drinks

4.1 How often do you usually eat the following kinds of foods? (Check the appropriate answer on each line)

	Seldom/ Never	1-3 times/ month	1-3 times/ week	4-6 times/ week	1-2 times/ day	>3 times/ day
Fruit/berries						
Cheese (all kinds)						
Potatoes						
Vegetables						
Fatty fish (e.g. salmon, trout, mackerel, herring)						
Gouda-type cheese						
Brown whey cheese						
Gamalost						
Liver paté						
Salami						
Ham						
Cured ham						
Mackerel in tomato						
Jam						
Caviar						
Mayonnaise-based sandwich salads						

4.2 To what degree have you changed your intake of the foods in 4.1 the last 3 months?

Not at all	Some	A lot

4.3 Do you eat some of the foods in 4.1 periodically?

Yes	No

If yes, which ones have you eaten a lot the last month?

4.4 What kind of fat do you use most often? (Check only one on each line)

	Butter	Hard margarine	Soft/light margarine	Oils	Do not use
On bread					
For cooking					

4.5 Do you take the following food supplements? (Check only one on each line)

	Yes, daily	Sometimes	No
Cod liver oil, cod liver oil capsules, fish oil capsules			
Vitamin and/or mineral supplements			

4.6 How much do you usually drink of the following?
(Check one per line).

	Seldom/never	1-6 glasses /week	1 glass/ day	2-3 glasses /day	>4 glasses /day
Whole milk, yoghurt					
Kefir					
Semi-skimmed milk, low fat yoghurt					
Cultura, Biola					
Skimmed milk (sour/sweet)					
Fruit juice					
Water					
Cola drinks					
Other fizzy drinks/thirst quenchers					

4.7 Do you usually drink fizzy drinks / cola?

With sugar	Without sugar

4.8 To what degree have you changed your intake of fizzy drinks in the last 3 months?

Not at all	Some	A lot

4.9 How many cups of coffee or tea do you drink daily?
(Write 0 if you do not drink coffee or tea daily)

Number cups coffee	
Number cups tea	

4.10 Do you normally salt your food?

Yes, a lot	Yes, some	No

4.11 How often have you consumed alcohol in the course of the past year?
(Low alcohol beer and non-alcoholic beer are not included)

4-7 times/ week	2-3 times /week	Once /week	2-3 times/ month	Once/ month	Few times /year	None past year	Never had alcohol

4.12 When you drink, do you usually drink: (Check more than one if applicable)

Beer	Wine	Spirits

4.13 To what degree have you changed your intake of alcohol in the last 3 months?

Not at all	Some	A lot

4.14 If you have changed your diet the last three months, how has it changed? (Check one or more)

Less fat	
More fat	
Less carbohydrates	
More carbohydrates	
More fruit and vegetables	
Less salt	
More fatty fish	
I have not made any changes	
Other	

5. Tobacco

5.1 Have you smoked/do you smoke daily?

Yes, currently	Yes, previously	Never

5.2 If you smoke daily now, or have smoked before

How many cigarettes do you or did you usually smoke daily?	
How many years altogether have you smoked?	

5.3 Have you used snus/do you use it daily?

Yes, currently	Yes, previously	Never

5.4 If you use snus daily now, or have used it previously:

How many snus do you or did you usually use daily?	
How many years altogether have you used snus?	

6. Education and work

6.1 How many years of schooling/education have you completed altogether?

	Years

6.2 What is your highest achieved education? (Check only one)

Primary and secondary school	
Upper secondary school	
College, 1 year	
College/university, 3 years (Bachelor)	
College/university, 5 years (Master)	
College/university, > 5 years	
PhD	

6.3 Are you currently employed?

Yes, full time	Yes, part time	No	Student

7. Physical activity

7.1 What kind of physical activity have you undertaken in you spare time in the course of the past year?
Estimate a weekly average for the year. From home to work is regarded as spare time. Answer both questions.

	Hours per week			
	None	Less than 1	1-2	3 or more
Light exercise You do not sweat or feel out of breath				
Hard physical activity You sweat and feel out of breath				

7.2 Describe the extent of movement and bodily exertion in your spare time. If the activity varies considerably, e.g. between summer and winter, then give an average. The question applies to the past year only.
(Check the appropriate answer)

Read, watch TV or other sedentary activity?	
Walk, cycle or move about in some other way at least 4 times/week (This should include walking or cycling to work, Sunday stroll/walk, etc.)	
Take part in physical exercise/sport, do heavy gardening work? (Note that the activity must take place at least 4 times a week)	
Exercise hard or take part in competitive sport regularly and several times a week	

8. Use of medicines

8.1 Do you take any of these medicines?

	Currently	Earlier	Never
Medicine for high blood pressure			
Cholesterol-reducing medicine			

8.2 If you have used any of the medicines in 8.1 in the last 4 weeks, give the name and reason for using them:

		How long have you used this medicine?	
Name of medicine	Reason for use	Up to 1 year	More than 1 year

9. Questions for women

9.1 Are you currently pregnant?

Yes	No	Not sure	Past fertile age

9.2 If you use the p-pill, mini-pill, p-injection, hormone loop or oestrogen;
which preparation do you use?

10. Other

Gender	
Age	
To be filled out by health personnel at inclusion in the trial (week 1)	
Height	
Weight	
Waist circumference	
Blood pressure	
Blood glucose	
Blood sample ID	
ID number	

Appendix 2

Supplementary Table 1. Mean change in blood pressure in each group, stratified by baseline blood pressure, paired samples t-test for change from start to end.

			Gamalost		Gouda-type cheese		Control		Study population	
			Mean	*P*	Mean	*P*	Mean	*P*	Mean	*P*
Systolic BP[1]	Hypertension	Baseline	151		152		153		152	
		Change midway	-6.67	0.030	-0.42	0.913	-10.94	<0.001	-6.71	<0.001
		Change end	-9.50	0.001	-3.58	0.120	-13.67	<0.001	-9.58	<0.001
		n	18		12		18		48	
	Normal high	Baseline	128		129		129		129	
		Change midway	-4.05	0.026	-0.54	0.727	0.29	0.871	-1.33	0.170
		Change end	-3.00	0.091	-3.54	0.049	-0.64	0.716	-2.44	0.016
		n	20		26		22		68	
	Optimal	Baseline	113		113		112		112	
		Change midway	-0.14	0.941	-0.40	0.900	3.38	0.208	0.66	0.642
		Change end	0.46	0.771	-3.50	0.101	0.25	0.913	-0.87	0.425
		n	13		10		8		32	
Diastolic BP	Hypertension	Baseline	92		92		92		92	
		Change midway	-4.06	0.008	-0.92	0.671	-5.06	0.020	-3.65	0.001
		Change end	-4.56	0.004	-0.75	0.691	-3.39	0.130	-3.17	0.004
		n	18		12		18		48	
	Normal high	Baseline	81		81		81		81	
		Change midway	-3.55	0.006	-1.35	0.186	-0.10	0.944	-1.61	0.020
		Change end	-2.20	0.038	-0.92	0.470	-0.05	0.976	-1.01	0.173
		n	20		26		22		68	
	Optimal	Baseline	73		71		71		72	
		Change midway	-3.71	0.051	1.30	0.526	-2.00	0.178	-1.72	0.119
		Change end	-1.54	0.336	-0.80	0.550	1.25	0.510	-0.58	0.519
		n	13		10		8		32	

[1]BP, Blood pressure

PAPER III

Effect of a high intake of cheese on cholesterol and metabolic syndrome: results of a randomized trial.

Author list: Nilsen, R.[1]*, Høstmark, A.T.[2], Haug, A.[3], Skeie, S.[1]

[1]Department of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences, Ås, Norway.

[2]Institute of Health and Society, University of Oslo, Oslo, Norway

[3]Department of Animal and Aquacultural Sciences, Norwegian University of Life Sciences, Ås, Norway.

*Corresponding author:

Rita Nilsen

P.O. Box 5003, 1432 Ås, Norway

Telephone: (+47) 6723 2532 Fax: (+47) 6496 5001

E-mail address: rita.nilsen@nmbu.no

Abstract

Background: Cheese is generally rich in saturated fat, which is associated with increased risk for cardiovascular diseases. Nevertheless, recent reports suggest that cheese may be anti-atherogenic.

Objective: The goal of this study was to assess whether intake of two types of Norwegian cheese, with widely varying fat and calcium content, might influence cardiovascular risk factors.

Design: 153 participants were randomized to one of three groups: Gamalost®, a traditional fat and salt free Norwegian cheese (50 g/day), Gouda-type cheese with 27% fat (80 g/day), and a control group with a limited cheese intake. Blood samples, anthropometric measurements, blood pressure and questionnaires about lifestyle and diet were obtained at inclusion and end.

Results: At baseline, there were no differences between the groups, mean age 43, 52.3% female. After 8 weeks intervention, there were no increases in total- or LDL cholesterol in the cheese groups compared to the control. Stratified analysis showed that those in the Gouda group with metabolic syndrome at baseline had significant reductions in total cholesterol at the end of the trial compared to control (-0.70 mmol/L, $p = 0.013$), and significantly higher reduction in mean triglycerides. In the Gamalost® group, those who had high total cholesterol at baseline had significantly reduction in total cholesterol compared to control (-0.40 mmol/L, $p = 0.035$).

Conclusions: In conclusion, cholesterol levels did not increase after high intake of 27% fat Gouda-type cheese over 8 weeks intervention, and stratified analysis showed that participants with metabolic syndrome had reduced cholesterol at the end of the trial.

Keywords: dairy, intervention, Gamalost, Gouda, cardiovascular diseases.

Introduction

Cardiovascular diseases (CVD) are the most common causes of mortality in the world (1) and lifestyle factors such as dietary changes are successful at reducing the risk of these diseases. Full fat dairy products, are rarely recommended in these so-called heart healthy diets due to the high content of saturated fat in those products, approximately 17% by weight in Norwegian Gouda-type cheeses (2), which is assumed to increase serum cholesterol levels. The Dietary Approaches to Stop Hypertension, for example, recommends a high intake of dairy products, with a focus on predominantly low-fat milk and yoghurt (3). In addition to serum cholesterol, raised serum triglyceride concentrations have long been associated with an increased risk for CVDs, however, whether it promotes CVD or is just a biomarker for risk is still debated (4). Even so, recommendations are to limit intake of saturated fats, or follow a Mediterranean style diet, to maintain or reduce serum triglyceride levels to below 1.7 mmol/L (4).

On the other hand, observational studies have shown that cheese intake is associated with lower serum triglycerides (5, 6). Furthermore, a higher intake of full fat dairy and total dairy was associated with a better cardiovascular health score than a low intake (7). Intervention trials have also shown that there is some difference within full fat dairy, as cheese intake was shown to lower LDL-cholesterol compared to butter intake of equal fat content (8, 9). Cheese and dairy products have also been associated with reduced prevalence (5) and incidence (6) of the metabolic syndrome, a cluster of risk factors for diabetes type 2 and CVD. The findings related to dairy and CVDs are, however, inconsistent and showing both a positive effect of cheese intake in women with decreased CVD risk (*p* for trend: 0.03) (10), a negative effect with a 32% higher risk in CVD mortality for each standard deviation increase in high fat dairy products (11), as well as a favorable cardiovascular risk profile in women, but not in men (12). The reasons for these inconsistencies could be several, including different study designs, different outcome measures, and whether they investigate dairy products separately or as a large group encompassing all dairy intake. Suggested mechanisms of action on the effect of dairy and cheese intake on serum lipids include the effect of bioactive compounds, fatty acids and micronutrients, specifically calcium (13), as well as inhibition of Δ9-desaturase activity through some unidentified cheese components, possibly related to conjugated linoleic acid (14).

We previously completed a cross-sectional trial to explore whether Gamalost® intake might influence factors of the metabolic syndrome. It was found that intake of Gamalost® was negatively associated with systolic blood pressure (BP) (B = -0.7, p = 0.03) (15). Since

Gamalost® is fat-free, we wanted to investigate experimentally whether intake of either Gamalost® or a Gouda-type cheese, would influence metabolic syndrome factors. Gouda-type cheeses are the most commonly consumed cheeses in Norway. Gamalost® is a traditional Norwegian skimmed milk cheese, unlike most other cheeses in that it is naturally free of salt and fat, contains only 160 mg calcium/100 g cheese and it has a high protein content and a high amount of bioactive peptides. Details on the production of Gamalost® have been previously described elsewhere (16). Norvegia®, the Gouda-type cheese included in this trial, contains 27% fat and 800 mg/100 g calcium, making it very different from Gamalost®. Since results on dairy intake and factors associated with metabolic syndrome have been inconsistent, and dairy fat content has been implicated, we wanted to compare the effects upon metabolic syndrome factors of these two widely differing cheeses. Possibly, variations in saturated fat, bioactive peptides and calcium between the cheeses may give different effects on metabolic syndrome factors.

The aim of this trial was accordingly to investigate whether intake of Norvegia® or Gamalost® cheese might influence factors of the metabolic syndrome, and if they influenced the factors differently.

Methods

Subjects

Participants in the trial were recruited from the general population, through local radio, newspapers and television. We specifically tried to recruit persons with moderately high BP, but normotensive persons were also included. Men and women over 18 years of age and who fluently read Norwegian were included. Exclusion criteria were pregnancy and use of blood pressure lowering medications.

Design

This randomized single-blinded controlled trial was performed with three parallel arms which is illustrated in figure 1. An eight week intervention period included measurements taken at baseline and at the end of the trial. The randomization procedure and envelopes containing information on which arm the participants had been allocated to were prepared by an independent person not involved in the study. Independent of all baseline measurements, the participants were handed the envelopes by two independent persons not involved in the study or the baseline measurements.

This study was carried out at the Department of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences, Ås, Norway from April 2013 to July 2013 and was approved by the Regional Committees for Medical and Health Research Ethics (Oslo, Norway) on 7th March, 2013 (2013/166) (registered at www.clinicaltrials.gov; NCT01913756). The study was conducted according to the guidelines laid down in the Declaration of Helsinki and written informed consent was obtained from all subjects.

Interventions

The participants were randomly assigned to one of three groups, either Norvegia®, Gamalost®, or control. Participants in the cheese groups were asked to maintain their habitual diet, whereas subjects in the control group were asked to limit their intake of the two intervention cheeses. The control group were given a list of cheeses they could consume, consisting mostly of fresh cheeses, blue cheese and cream cheese. Norvegia® and Gamalost® are registered trademarks of TINE SA, Norway. The participants consumed 50 g/day or 80 g/day of Gamalost® or Norvegia®, respectively. These amounts were chosen because they were judged to be higher than the average intake of each cheese, but not so high that the participants were unable to

consume the designated amount. Also, in order to have similar cheese protein intakes in the two cheese groups, the Norvegia® intervention cheese amount was larger than the Gamalost® intervention. The participants were equipped with digital kitchen scales to accurately weigh out the daily intake. The Gamalost® cheeses were all made from the same batch and they were ripened for 10 days. The Norvegia® cheeses were also from the same batch, and they were ripened for approximately 90 days. The nutritional value of the cheeses are presented in table 1.

Compliance with the cheese intake was judged by evaluation of charts of weighed daily cheese intake, filled out by a subset of the study population.

Questionnaire

A questionnaire was developed for a cross-sectional trial on Gamalost® intake and blood pressure that preceded the current study (15). The questionnaire was a revised version of the previously validated questionnaires used in the cross-sectional Oslo Health Study (the main questionnaire and the second supplementary questionnaire 1 of the Oslo Health Study were used) (17). The baseline questionnaire contained questions about lifestyle, health, medication use, and habitual diet. Some questions focused specifically on dairy product intake. Total dairy product intake was calculated by summarizing the frequency of intake of all cheese, all milk, and fermented milk products. The exclusion questionnaire focused on diet through the trial, difficulty with following the diet, and whether the participants had experienced any discomfort during the intervention. A version of the baseline questionnaire translated into English can be found in supplementary material 1.

Blood samples

Venous blood samples were drawn in the morning between 06:30 and 10:30 after an overnight fast (approximately 10-12 hours), using the Vacutainer® system (Becton Dickinson Co., Franklin Lakes, NJ, USA). The samples were centrifuged at 2500 rpm for 10 minutes in room temperature and the serum was separated approximately one to two hours after the blood was drawn. The serum was frozen to -20°C within five hours. Fürst Medical Laboratories (Oslo, Norway) conducted the serum analyses. The measured biochemical markers were total cholesterol (mmol/L), HDL cholesterol (mmol/L), LDL cholesterol (mmol/L) and triglycerides (mmol/L). Fasting blood glucose (mmol/L) was measured in capillary blood by the finger stick method, using a LifeScan OneTouch® Verio™Pro (Cilag GmbH International, Switzerland).

159

160 Blood pressure

161 Blood pressure was measured using a Microlife® BP A200 sphygmomanometer (Microlife,
162 Widnau, Switzerland). BP was measured after approximately 10 minutes of rest, in a sitting
163 position and according to the American Heart Association guidelines (18). Three consecutive
164 measurements were taken and the average of the second and third measurements were
165 recorded. All participants were informed of their BP and whether or not it was within the
166 normal range.

167

168 Anthropometric measurements

169 Body weight was measured without shoes or heavy clothing, to the nearest 0.1 kg using digital
170 scales (TBF-300A Body Composition Analyzer, Tanita, Tokyo, Japan). Height was measured
171 to the nearest 0.1 cm using a portable stadiometer (Seca 217, Seca, Hamburg, Germany). Body
172 mass index was calculated as weight (kg) divided by the square of height (m). Waist
173 circumference was measured using a measuring tape (Seca 201 Circumference measuring tape,
174 Seca) to the nearest 0.1 cm, according to World Health Organization recommendations, i.e. at
175 the midpoint between the iliac crest and the lowest rib margin (19).

176

177 Metabolic syndrome

178 In order to qualify as having metabolic syndrome, a person must have at least three of the
179 following five criteria: elevated waist circumference (country-specific cut points, ≥94 cm and
180 ≥80 cm for European men and women, respectively), elevated triglycerides (≥1.7 mmol/L),
181 reduced HDL-cholesterol (<1.0 mmol/L in men and <1.3 mmol/L in women), raised BP
182 (systolic ≥130 and/or diastolic ≥85 mmHg), or elevated fasting blood glucose (≥5.6 mmol/L)
183 (20). Participants were stratified into two groups for some statistical analyses: MetS-yes if they
184 had metabolic syndrome at baseline, and MetS-no if they did not meet the criteria at baseline.
185 They were also stratified for subgroup analyses by the presence of each individual MetS factor
186 at baseline and categorized as follows: waist-yes/waist-no, TAG-yes/TAG-no, GLU-yes/GLU-
187 no, HDL-yes/HDL-no, SBP-yes/SBP-no, and DBP-yes/DBP-no. Total and LDL-cholesterol
188 are not part of the metabolic syndrome and were therefore stratified based on cholesterol
189 guidelines from "Adult Treatment Panel III" (21). Hence, LDL-yes/LDL-no with cut-off at 3.4
190 mmol/L and CHOL-yes/CHOL-no with cut-off at 5.2 mmol/L.

191

192 Statistical analysis

193 Statistical analyses were performed using SPSS 21.0 (IBM Corporation, Armonk, New York).
194 Prior to analyses, the dataset was recoded by an independent person so that the primary
195 researcher was blinded in regards to intervention group. Data was analyzed according to the
196 intention to treat principle. Baseline characteristics of the study population are presented as
197 mean (standard deviation), or as percentages were appropriate. One-way ANOVA with
198 Bonferroni correction for multiple comparisons or the chi-square (χ^2) test were used to assess
199 differences between intervention groups at baseline. Paired samples t-test was used to assess
200 change in metabolic syndrome factors from inclusion to end of trial in each intervention group.
201 The Dunnett test was used to evaluate mean changes between each treatment group and the
202 control group. The Dunnett test was also done for the groups stratified by MetS-yes or no, and
203 by each individual factor of the syndrome, as well as total and LDL-cholesterol. A *p*-value
204 <0.05 was considered statistically significant.
205

Results

Baseline characteristics

At inclusion 153 participants were randomized to one of the three groups of the trial (n = 50 in Norvegia® group, n = 53 in Gamalost® group, n = 50 in control group). Five participants were lost to follow-up, one in the Norvegia® group and two each in the Gamalost® and control groups, as illustrated in figure 1. Two participants in the Norvegia® group lacked some of the baseline or follow-up measurements due to failure to fast (n = 1) or failure to complete blood draw (n = 1), resulting in an effective sample size of 47 for that group. The baseline characteristics of the whole study population and the three groups are presented in table 2. Approximately 30% of the population met the criteria to be diagnosed with the metabolic syndrome. As can be seen, there were no major differences in dairy intake or factor of the metabolic syndrome between the three groups at inclusion. LDL-cholesterol was higher in the control group than the two other groups (p = 0.05). As expected from normal Norwegian consumption patterns, the participants had a higher intake of all Gouda-type cheeses (approximately six servings per week) than Gamalost® (less than 1 serving per week).

As can be seen in table 3, all the individual metabolic syndrome variables were strongly correlated with the whole syndrome (p <0.001), but systolic BP, diastolic BP and waist circumference had slightly stronger correlations than the other factors. As expected, all factors were positively correlated with metabolic syndrome, except HDL cholesterol which had a negative correlation. Table 4 shows the prevalence of each metabolic syndrome factor in those participants who are categorized with metabolic syndrome. Over 90 % of participants who met the criteria for the metabolic syndrome had systolic BP over 130 mmHg, 30% had high triglycerides, whereas just 11 % of the participants met the low HDL-cholesterol criteria.

Total and LDL-cholesterol changes

As shown in table 5, a paired samples t-test showed that total cholesterol decreased significantly in the entire study population during the intervention, but analyzing the three groups separately, cholesterol was only significantly decreased in the Norvegia® group (-0.204 mmol/L, p = 0.017). Table 6 shows that when stratifying by metabolic syndrome diagnosis, total cholesterol was reduced in MetS-yes participants in Norvegia® group (-0.70 mmol/L, p <0.001). Those participants who had high total cholesterol at baseline (table 7) had significant decreases in total cholesterol in both the Norvegia® (-0.39 mmol/L, p = 0.021) and the Gamalost® groups (-0.39 mmol/L, p = 0.001). Comparing with the control group, total

cholesterol was only significantly reduced in the Norvegia® group (table 8): for those with MetS-yes, cholesterol was lowered by 0.70 mmol/L (p = 0.013). Table 8 shows that in those participants who had high total cholesterol at baseline, total cholesterol was decreased in both the Norvegia® (-0.39 mmol/L, p = 0.021) and Gamalost® groups (-0.40 mmol/L, p = 0.035) compared to control.

LDL-cholesterol was reduced in the whole study population for participants who had high LDL at baseline (table 7) (-0.17 mmol/L, p = 0.025), but this was only found in the Gamalost® group (-0.32 mmol/L, p = 0.011) when separating the groups. There was no effect on LDL-cholesterol when comparing with the control group.

Metabolic syndrome changes

There were no overall effects of the cheese interventions on the metabolic syndrome as a whole (data not shown), but there were some changes in the individual factors. When stratifying participants by the presence or absence of the metabolic syndrome at baseline, the paired samples t-test and the Dunnett test showed some differences in whether or not the participants met the metabolic syndrome criteria. When comparing the change in each metabolic syndrome variable between the intervention groups with the control group, there were no differences between the cheese groups and the control group when analyzing all the participants in each group (data not shown), but again, the stratified analyses showed some changes.

As can be seen from table 5, paired samples t-test showed that waist circumference decreased significantly in the entire study population and in the three groups separately during the intervention. Table 8 shows that MetS-yes participants in the Gamalost® group borderline significantly reduced their waist circumference compared to the control group (-2.0 cm, p = 0.054). Waist circumference was also significantly decreased in the Gamalost® group for those participants with waist-yes compared to the control group (-2.0, p = 0.037).

As shown in table 5, there was a slight significant overall increase in fasting blood glucose in the whole study population (p = 0.049) which was only borderline significantly present in the Gamalost® group when analyzing the three groups separately. There was no significant effect on glucose change when comparing the cheese intervention groups with the control group (data not shown). As can be seen from table 5, paired samples t-test showed that blood pressure decreased significantly in the entire study population during the intervention. All three intervention groups obtained significantly decreased systolic BP during intervention, whereas

the Gamalost® group was the only group with significant decrease in diastolic BP. There were no differences in systolic or diastolic BP when comparing the cheese groups with the control group at eight weeks (Author, 2015, unpublished observations). For participants who were MetS-no there were some changes in metabolic syndrome variables, as seen in supplementary material 2, but there were no significant differences between the cheese groups and the control group.

Serum triglycerides decreased in the Norvegia® group for MetS-yes participants (table 6) (-0.29 mmol/L, $p = 0.039$). As shown in table 8, compared to the control group those participants who were MetS-yes, significant reductions in triglycerides (-0.70 mmol/L, $p = 0.047$) were measured in the Norvegia® group. As can be seen from table 5, there was a slight significant overall decrease in HDL-cholesterol ($p = 0.004$) in the study population which was only present in the Gamalost® group when analyzing the three groups separately. However, this association was lost when comparing the Gamalost® group with the control group (results not shown).

Discussion

The results of this randomized controlled trial suggest a neutral effect on the metabolic syndrome as well as serum cholesterol in participants who consumed a moderate to large amount of the cheeses Norvegia® and Gamalost®, compared to a control group. When participants were stratified, i.e. by MetS at baseline and by each factor of MetS at baseline, the results showed some changes in cholesterol and triglycerides according to cheese intervention group.

Cholesterol and cheese intake

Total serum cholesterol is not part of the diagnostic criteria for metabolic syndrome, however it was included in this trial due to its possible relationship with CVDs. The American Heart Association's diet and lifestyle recommendations to prevent CVD make two recommendations related to cheese intake: 1) select fat-free, 1 % fat and low-fat dairy products, and: 2) to lower cholesterol, reduce saturated fat to no more than 5-6 % of total calories, about 13 grams on a 2000 kcal/day diet (22). In this trial, the participants in the Norvegia® group consumed about 14 grams/day of saturated fat just from the cheese, but at the end of follow-up there were no increases in total or LDL-cholesterol after eight weeks of increased cheese consumption. Furthermore, those participants in the Norvegia® group who were MetS-yes and those who had high cholesterol at baseline had reduced their total cholesterol levels from baseline to the end of the trial, which was also found to be significant when comparing the Norvegia® group to the control group of low cheese intake. We are not aware of many similar intervention trials investigating the effect of different cheeses on cholesterol levels, but some results are in accordance with ours and indicate that cheese may not raise cholesterol, as the previously stated recommendations would suggest. Results from an Iranian cross-sectional trial showed that those who consumed cheese more than 7 times/week did not have increased cholesterol compared to those who consumed cheese less than 7 times/week (23). They also found lower odds of having metabolic syndrome and low HDL-cholesterol if participants had a high cheese intake. However, a cross-sectional trial of adolescents in Portugal found that total cholesterol was borderline significantly higher in the appropriate cheese intake group compared to the low cheese intake group (24). Results from the National Health and Nutrition Examination Survey (NHANES) III show no association between cheese intake and total cholesterol levels in men or women in the U.S., but higher frequency of cheese intake was associated with higher HDL-cholesterol in women only (12). It is difficult, however, to compare these trials as they are from

different countries and habitual diets are likely dissimilar between the three. The three previously mentioned trials were conducted in Iran, Portugal and the U.S, respectively, which are countries with dietary patterns that are distinct from each other. Annual consumption per capita figures show that the average cheese intake in Iran is 4.9 kg (2013), in Portugal it is 9.6 kg (2012) while it is 15.4 kg (2013) in the US (25, 26). In Norway the annual consumption was higher than the previously mentioned countries, with 18.1 kg in 2013 (26), or approximately 7.5 servings per week as measured in the current trial.

These contradictory results from cross-sectional studies indicate the need for intervention trials investigating the effect of dairy and cheese on cholesterol under differing habitual diets. Not many intervention trials compare a high cheese intake with a control group of low cheese intake, making it difficult to compare our results with other populations. However, there are some similar trials which show a comparable effect on cholesterol. Total cholesterol was significantly lower on a cheese diet (150 g/8 MJ daily) compared to a diet of butter and casein (27) and a high cheese diet (143 g/day) resulted in 5.7 % lower total cholesterol compared to a butter diet (47 g/day) (8). Tholstrup *et al.* (2004) investigated the effect of 205 g hard cheese/10 MJ daily compared to butter and milk intake, and found a moderately lower LDL-cholesterol after the cheese intervention compared to butter intervention (9). They found no significant effect on total cholesterol which was 0.20 mmol/L higher after the butter intervention compared to cheese intervention ($p = 0.054$). It is not completely clear why we have these neutral effects or reductions in cholesterol on a high cheese or high dairy diet. It has been suggested that the main mechanism of action is through calcium, which binds to saturated fatty acids and forms insoluble salts which increases fecal fat excretion, making less saturated fat available for absorption (28). A meta-analysis of randomized controlled trials indicated that increasing calcium intake from dairy by 1241 mg/day corresponded to an increase of 5.2 g/day of fecal fat excretion (29). A randomized crossover intervention study of 15 healthy men with a 14 day dietary intervention of increased calcium from milk (1143 mg Ca/10 MJ) or cheese (1172 mg Ca/10 MJ), or low calcium control group (362 mg Ca/10 MJ) was carried out in Copenhagen from 2011 to 2012 (30). Feces and urine were collected at days 10-14 and 14, respectively, and analyzed for fat and calcium content. Contrary to our results, total and LDL cholesterol increased from baseline in all three groups. However, this effect was attenuated in the cheese and milk groups compared to control. Fecal fat was increased in both milk and cheese groups, and this was correlated with change in both LDL and total cholesterol. In our trial the Norvegia® group consumed 640 mg/day of calcium just from the cheese, indicating that increased calcium intake could be one of the reasons why total cholesterol did not increase

even though the participants increased their cheese intake. However, the Gamalost® group only consumed 80 mg/day calcium from the cheese, but still had a reduction in total cholesterol compared to control in those participants who had high cholesterol at baseline. This could be a random effect, or it indicates some other mechanism by which cheese may be hypocholesterolemic, e.g. possibly related to the presence of bioactive peptides, but further investigations are necessary to support this hypothesis.

The amount of total cholesterol reduction in Met-S yes participants in our trial, about 0.7 mmol/L in the Norvegia® compared to control group, may be of clinical significance. A meta-analysis estimated that each 1 mmol/L reduction in total cholesterol corresponded to a 17.5% reduction in relative risk of all-cause mortality (31), hence a reduction of 0.7 mmol/L could contribute to reductions in mortality. Our results show no effect of Norvegia® on LDL- or HDL-cholesterol separately. In the Gamalost® group, there was a small but significant decrease in HDL-cholesterol compared to baseline in both those who were MetS-no and those who were HDL-no, however, this effect was not present when comparing Gamalost® to the control group.

Metabolic syndrome and cheese intake

Hypertension is a very prevalent condition around the world and it was estimated that up to 17% of all deaths are attributed to high BP (32). In this trial, over 90% of participants with metabolic syndrome had higher than normal systolic BP, making it the most prevalent criteria of the syndrome. However, after eight weeks of intervention there were no significant effects of the cheeses on BP in this trial. Several studies have shown positive effects of dairy product intake on MetS or single factors of the MetS. However, there are inconsistencies and variations in study design and in which dairy products are studied. In the observational Oslo Health Study, results showed that the frequency of cheese intake was significantly negatively associated with serum triglycerides, diastolic BP and waist circumference, and positively associated with HDL-cholesterol (5). Similarly, a French prospective observational study found that frequency of cheese intake was negatively associated with triglyceride levels and also lower increase in waist circumference over nine years (6). Another French prospective observational trial found no effect of cheese intake on factors of MetS in men or women, however, when stratifying by baseline BMI, cheese intake was significantly positively associated with HDL-cholesterol and negatively with fasting glucose in those who had a BMI <25 kg/m^2 (33). These trials did not differentiate between different types of cheese, meaning the results could be associated with any kind of cheese. Only about one third of the MetS-yes participants in our trial had high

triglycerides, making it one of the least prevalent abnormalities. It has been known for a long time that serum triglycerides is associated with CVD risk, independent of other risk factors (34), but the effect of dietary change on triglycerides is less well established. A meta-analysis of randomized controlled trials found that low-fat diets had no effect on serum triglycerides in women (35) and neither did low glycemic index diets (36).

There are no good figures for the prevalence of metabolic syndrome in the general healthy Norwegian population. The International Diabetes Federation estimates that about 25% of the world's population have metabolic syndrome (37), whereas the prevalence in healthy non-diabetic Europeans was approximately 15% in 2004 (38). In the current trial, about one third of the population met the criteria, indicating that the prevalence has either increased over the last 10 years, or that our population is not representative of the general European population. However, the different metabolic syndrome definitions used can also influence the prevalence, as the European trial used a modified WHO definition where hyperinsulinemia had to be present in order to be diagnosed. Furthermore, we specifically tried to recruit participants with moderately high BP, which obviously has an effect on the prevalence of metabolic syndrome in this study.

The design of the study itself is the main strength of this trial. The duration of the intervention was quite long and the population fairly large compared to similar trials, and the randomization allowed for three groups of similar characteristics at baseline. The Norvegia® intervention cheese is the most commonly consumed cheese in Norway, making the results relevant to a large part of the population.

In conclusion, even though cheese and high-fat dairy products are not recommended in heart-healthy diets, results from this trial do not show a negative effect of cheese intake on cholesterol or metabolic syndrome. Consuming 80 g/day of Norvegia®, a 27% fat Gouda-type cheese, appeared to have a slight hypocholesterolemic effect in those participants who had metabolic syndrome and high cholesterol at baseline, compared to the control group of low cheese intake. Additional studies are needed to confirm these results, as well as to investigate the effect of other cheeses.

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The authors' contributions are as follows: all authors contributed to formulating the research question, designing the study, carrying out the statistical analysis and critically revising the manuscript. Additionally, R. N. carried out the study and drafted the manuscript.

None of the authors have any conflicts of interest to declare.

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Tables

Table 1. Nutrient composition (per 100 g) of intervention cheeses

Nutrient	Gamalost® (50 g/day)	Norvegia® (80 g/day)
Energy[1], kcal	213 (107)	351 (281)
Protein[1], g	50 (25)	27 (22)
Fat[1], g	1 (0.5)	27 (22)
Saturated, g	0 (0)	17 (14)
Carbohydrates[1], g	1 (0.5)	0 (0)
Calcium[1], mg	160 (80)	800 (640)
Sodium[1], mg	24 (12)	402 (322)
Magnesium[1], mg	13 (6.5)	33 (26)
Potassium[1], mg	98 (49)	77 (62)

[1]From TINE SA, manufacturer of Gamalost® and Norvegia®

Table 2. Baseline characteristics (mean (SD) or %) for all participants and by intervention groups.

	Intervention group								
	All (*n*=153)		Norvegia® (*n*=50)		Gamalost® (*n*=53)		Control (*n*=50)		
Characteristic	Mean	SD[1]	Mean	SD	Mean	SD	Mean	SD	*p*
Gender, female (%)	52.3		60.0		50.9		46.0		0.4
Age (years)	43.1	16.4	42.7	15.8	41.2	17.0	45.5	16.4	0.4
Weight (kg)	77.2	14.8	76.0	13.6	75.6	13.7	79.9	16.8	0.3
Height (cm)	173.9	8.9	171.9	8.7	174.9	8.7	174.7	9.4	0.2
BMI (kg/m^2)	25.7	3.7	25.6	3.5	24.6	3.3	26.0	3.7	0.1
Waist circumference (cm)	83.1	11.8	82.8	10.9	80.9	11.3	85.8	12.9	0.1
Systolic BP (mmHg)	132.3	17.2	130.6	14.7	131.5	19.3	134.8	17.2	0.4
Diastolic BP (mmHg)	82.4	9.8	81.4	8.9	82.5	10.6	83.1	10.0	0.7
Total cholesterol (mmol/L)	5.2	1.1	5.3	1.2	5.0	1.2	5.4	1.0	0.2
LDL cholesterol (mmol/L)	2.9	1.0	2.9	1.0	2.7	0.9	3.1	0.9	0.05
HDL cholesterol (mmol/L)	1.7	0.4	1.7	0.4	1.7	0.5	1.6	0.5	0.6
Triglycerides (mmol/L)	1.1	0.6	1.1	0.8	1.0	0.6	1.2	0.5	0.7
Blood glucose (mmol/L)	5.8	0.7	5.7	0.6	5.7	0.9	5.8	0.5	0.7
Metabolic syndrome (%)	30.1		32.0		24.5		34.0		0.5
Education (years)	16.6	2.9	17.0	2.3	16.6	2.7	16.4	3.5	0.6
Smoking[2] (%)	3.3		2.0		3.8		4.1		0.9
Physical activity[3] (%)	38.4		36.7		39.6		38.8		0.9
Total dairy[4]	18.4	11.9	17.5	10.1	19.7	12.9	18.0	12.7	0.6
Total cheese[4]	7.5	4.6	7.1	4.2	8.0	4.7	7.2	4.9	0.5
Gouda-type cheeses[4]	5.7	4.3	5.4	3.6	6.1	4.6	5.6	4.8	0.7
Gamalost®[4]	0.7	1.9	0.6	1.8	0.6	1.7	0.8	2.2	0.9

[1]SD, standard deviation

[2]Percentage daily smokers

[3]Percentage who reported moderate to hard physical activity more than four hours per week

[4]Servings per week

Table 3. Pearson correlations (2-tailed) between individual metabolic syndrome variables and the whole metabolic syndrome at baseline, within the whole study population (n = 153).

Variable	Metabolic syndrome	p
Waist circumference	0.663	<0.001
Systolic BP[1]	0.663	<0.001
Diastolic BP	0.637	<0.001
Triglycerides	0.467	<0.001
HDL-cholesterol	-0.274	0.001
Blood glucose	0.528	<0.001

[1]BP, blood pressure

Table 4. The prevalence of each metabolic syndrome variable within participants in the whole study population who are metS-yes (*n* = 46).

Variable	Percentage
High systolic BP[1]	93.5%
High blood glucose	87.0%
High diastolic BP	78.3%
High waist circumference	76.1%
High triglycerides	30.4%
Low HDL-cholesterol	10.9%

[1]BP, blood pressure

Table 5. Mean difference (MD)* with 95% CI in each group and the whole study population, paired samples t-test comparing start to 8 weeks follow-up.

	Norvegia®			Gamalost®			Control			Study population		
Variable	MD	95% CI	*p*	MD	95% CI	*p*	MD	95% CI	*p*	MD	95% CI	*p*
Total chol, mmol/L	-0.20	-0.37, -0.04	0.017	-0.09	-0.23, 0.05	0.215	-0.07	-0.25, 0.12	0.477	-0.12	-0.21, -0.03	0.013
LDL-chol, mmol/L	-0.07	-0.21, 0.07	0.342	0.00	-0.12, 0.11	0.959	-0.07	-0.21, 0.07	0.292	-0.05	-0.12, 0.03	0.212
TAG, mmol/L	-0.06	-0.18, 0.06	0.324	0.02	-0.08, 0.12	0.751	0.13	-0.10, 0.36	0.259	0.03	-0.06, 0.12	0.530
Waist, cm	-1.0	-1.5, -0.4	0.001	-1.6	-2.1, -1.1	<0.001	-1.0	-1.6, -0.5	0.001	-1.2	-1.5, -0.9	<0.001
Systolic BP[1], mmHg	-3.5	-5.8, -1.3	0.003	-4.4	-7.0, -1.8	0.001	-5.4	-8.5, -2.2	0.001	-4.4	-6.0, -2.9	<0.001
Diastolic BP, mmHg	-0.9	-2.6, 0.9	0.319	-2.9	-4.4, -1.4	<0.001	-1.1	-3.3, 1.1	0.333	-1.6	-2.7, -0.6	0.002
Glucose, mmol/L	0.09	-0.11, 0.28	0.368	0.20	0.00, 0.41	0.051	0.05	-0.16, 0.26	0.634	0.12	0.00, 0.23	0.049
HDL-chol, mmol/L	-0.04	-0.10, 0.03	0.299	-0.06	-0.10, -0.01	0.010	-0.04	-0.10, 0.01	0.100	-0.05	-0.08, -0.01	0.004

*For baseline values, see Table 2.

[1]BP, blood pressure

Table 6. Paired samples t-test stratified by positive metabolic syndrome diagnosis (MetS-yes) in each group and the whole study population at baseline.*

		Norvegia® (n = 15)			Gamalost® (n = 13)			Control (n = 16)			Study population (n = 44)		
Variable		Mean	95% CI	*p*	Mean	95% CI	*p*	Mean	95% CI	*p*	Mean	95% CI	*p*
Total cholesterol	Baseline	6.08			5.25			5.31			5.56		
	Change	-0.59	-0.86, -0.31	<0.001	-0.03	-0.35, 0.29	0.840	0.11	-0.37, 0.58	0.639	-0.17	-0.39, 0.05	0.128
Triglycerides	Baseline	1.77			1.25			1.43			1.49		
	Change	-0.29	-0.56, -0.02	0.039	-0.13	-0.36, 0.11	0.263	0.41	-0.26, 1.07	0.212	0.01	-0.25, 0.28	0.927
Waist circumference	Baseline	90.7			90.5			97.4			93.1		
	Change	-1.3	-2.8, 0.1	0.073	-1.9	-3.1, 0.8	0.004	0.1	-1.2, 1.4	0.895	-1.0	-1.7, -0.2	0.011
Systolic BP[1]	Baseline	141.9			150.9			150.1			147.5		
	Change	-1.8	-7.3, 3.7	0.493	-8.3	-15.9, -0.7	0.035	-10.3	-17.5, -3.1	0.008	-6.8	-10.6, -3.1	0.001
HDL-cholesterol	Baseline	1.67			1.58			1.40			1.54		
	Change	-0.03	-0.14, 0.08	0.535	-0.05	-0.13, 0.02	0.148	-0.07	-0.15, 0.02	0.107	-0.05	-0.10, -0.00	0.039

* Only factors with significant associations are shown.

[1]BP, blood pressure

Table 7. Paired samples t-test stratified by the presence of each metabolic syndrome factor in each group and the whole study population at baseline.*

		Norvegia®			Gamalost®			Control			Study population		
Variable		Mean	95% CI	*p*	Mean	95% CI	*p*	Mean	95% CI	*p*	Mean	95% CI	*p*
Total cholesterol	n	28			20			27			75		
	Baseline	6.04			6.14			6.00			6.17		
	Change	-0.39	-0.62, -0.15	0.002	-0.39	-0.61, -0.17	0.001	0.007	-0.22, 0.23	0.947	-0.25	-0.38, -0.11	0.001
LDL-cholesterol	n	13			11			16			40		
	Baseline	4.16			3.96			4.01			4.05		
	Change	-0.20	-0.55, 0.15	0.245	-0.32	-0.56, -0.09	0.011	-0.05	-0.28, 0.18	0.655	-0.17	-0.32, -0.02	0.025
Triglycerides	n	7			5			6			18		
	Baseline	2.62			2.54			2.08			2.42		
	Change	-0.48	-1.09, 0.13	0.104	-0.64	-0.86, -0.41	0.001	-0.51	-1.33, 0.32	0.175	-0.53	-0.82, -0.24	0.001
Waist circumference	n	15			15			18			48		
	Baseline	92.2			94.0			99.2			94.2		
	Change	-0.9	-2.2, 0.5	0.200	-2.4	-3.3, -1.5	<0.001	-0.4	-1.7, 0.9	0.493	-1.2	-1.9, -0.5	0.001
Systolic BP[1]	n	25			25			28			78		
	Baseline	141.8			146.8			145.9			144.9		
	Change	-3.5	-6.7, -0.4	0.030	-8.4	-12.4, -4.3	<0.001	-9.0	-13.5, -4.6	<0.001	-7.1	-9.3, -4.8	<0.001
Diastolic BP	n	15			19			16			50		
	Baseline	91.7			39.4			94.4			93.2		
	Change	-3.0	-7.3, 1.3	0.156	-5.7	-8.7, -2.7	0.001	-4.1	-7.7, -0.6	0.026	-4.4	-6.3, -2.4	<0.001

* Only factors with significant associations are shown.

[1]BP, blood pressure

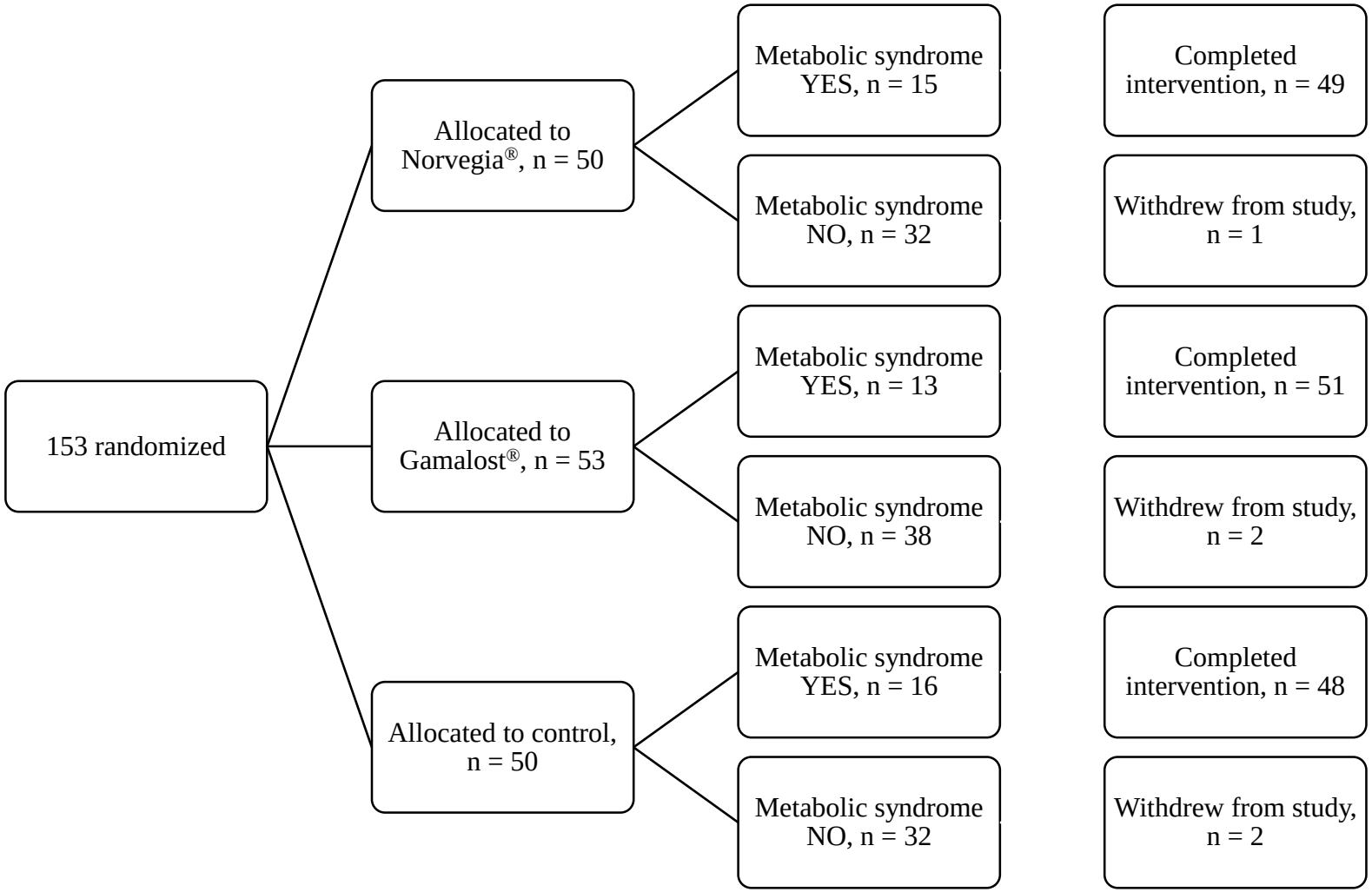
Table 8. Stratified analysis of significant changes* by MetS (yes or no) or by individual factors of MetS and total cholesterol (yes or no), comparing control group with the two cheese diets. Values are mean (SE), 2-sided *p*-values for the difference from control (Dunnett test).

	Baseline mean	End mean	Change	Difference from control (95% CI)	*p*
MetS-yes					
Tot chol (mmol/L)					
Control	5.45 (0.23)	5.41 (0.29)	0.11 (0.22)		
Norvegia®	6.01 (0.33)	5.49 (0.29)	-0.59 (0.13)	-0.70 (-1.25, -0.14)	0.013
Gamalost®	5.25 (0.33)	5.22 (0.22)	-0.03 (0.15)	-0.14 (-0.72, 0.44)	0.813
TAG (mmol/L)					
Control	1.46 (0.14)	1.84 (0.30)	0.41 (0.31)		
Norvegia®	1.71 (0.27)	1.48 (0.21)	-0.29 (0.13)	-0.70 (-1.38, -0.01)	0.047
Gamalost®	1.25 (0.17)	1.12 (0.11)	-0.13 (0.11)	-0.53 (-1.25, 0.18)	0.168
Waist (cm)					
Control	97.6 (2.1)	97.5 (2.2)	0.08 (0.6)		
Norvegia®	91.5 (2.6)	89.4 (2.8)	-1.32 (0.68)	-1.40 (-3.35, 0.54)	0.186
Gamalost®	90.5 (3.2)	88.6 (3.2)	-1.91 (0.53)	-1.99 (-4.01, 0.03)	0.054
Individual factors					
Tot chol-yes (mmol/L)					
Control	6.06 (0.14)	6.00 (0.17)	0.01 (0.11)		
Norvegia®	6.03 (0.19)	5.66 (0.20)	-0.39 (0.11)	-0.39 (-0.73, -0.05)	0.021
Gamalost®	6.11 (0.18)	5.75 (0.14)	-0.39 (0.10)	-0.40 (-0.77, 0.02)	0.035
Waist-yes (cm)					
Control	99.3 (1.75)	98.8 (1.80)	-0.43 (0.62)		
Norvegia®	92.3 (2.28)	91.3 (2.35)	-0.85 (0.63)	-0.42 (-2.27, 1.43)	0.828
Gamalost®	94.0 (2.11)	91.7 (2.27)	-2.39 (0.43)	-1.95 (-3.80, -0.10)	0.037

*Only factors with significant associations are shown.

Figure legends

Figure 1. Flow chart of a single-blinded, randomized, controlled trial of Gamalost® and Norvegia® in 153 participants.


153 randomized
Allocated to Norvegia®, n = 50
Allocated to Gamalost®, n = 53
Allocated to control, n = 50
Metabolic syndrome YES, n = 15
Metabolic syndrome NO, n = 32
Metabolic syndrome YES, n = 13
Metabolic syndrome NO, n = 38
Metabolic syndrome YES, n = 16
Metabolic syndrome NO, n = 32
Completed intervention, n = 49
Withdrew from study, n = 1
Completed intervention, n = 51
Withdrew from study, n = 2
Completed intervention, n = 48
Withdrew from study, n = 2

Supplementary materials

Supplementary material 1. Questionnaire at inclusion

1. Your health

1.1 How would you describe your present state of health? (Check only one answer)

Poor	Not very good	Good	Very good

1.2 Do you have any of these illnesses, or have you suffered from of them in the past?

	Yes	No	Age on first occasion
Asthma			
Chronic bronchitis/emphysema			
Diabetes			
Osteoporosis			
Myocardial infarction			
Angina pectoris (cardiac spasm)			
Stroke/cerebral haemorrhage (“drip”)			
High blood pressure			

2. Where you grew up/where you live

2.1 Where did you live for most of the time before you reached the age of 16 years? (Check one alternative and specify)

Same place	
Another county in Norway	County:
Outside Norway	Country:

2.2 Have you moved in the course of the last five years? (Check only one answer)

No	Yes, once	Yes, several times

3. Weight

3.1 Assess your weight when you were 25 years | | kg | old:

4. Food and drinks

4.1 How often do you usually eat the following kinds of foods? (Check the appropriate answer on each line)

	Seldom/ Never	1-3 times/ month	1-3 times/ week	4-6 times/ week	1-2 times/ day	>3 times/ day
Fruit/berries						
Cheese (all kinds)						
Potatoes						
Vegetables						
Fatty fish (e.g. salmon, trout, mackerel, herring)						
Gouda-type cheese						
Brown whey cheese						
Gamalost						
Liver paté						
Salami						
Ham						
Cured ham						
Mackerel in tomato						
Jam						
Caviar						
Mayonnaise-based sandwich salads						

4.2 To what degree have you changed your intake of the foods in 4.1 the last 3 months?

Not at all	Some	A lot

4.3 Do you eat some of the foods in 4.1 periodically?

Yes	No

If yes, which ones have you eaten a lot the last month?

4.4 What kind of fat do you use most often? (Check only one on each line)

	Butter	Hard margarine	Soft/light margarine	Oils	Do not use
On bread					
For cooking					

4.5 Do you take the following food supplements? (Check only one on each line)

	Yes, daily	Sometimes	No
Cod liver oil, cod liver oil capsules, fish oil capsules			
Vitamin and/or mineral supplements			

4.6 How much do you usually drink of the following?
(Check one per line).

	Seldom /never	1-6 glasses /week	1 glass/ day	2-3 glasses /day	>4 glasses /day
Whole milk, yoghurt					
Kefir					
Semi-skimmed milk, low fat yoghurt					
Cultura, Biola					
Skimmed milk (sour/sweet)					
Fruit juice					
Water					
Cola drinks					
Other fizzy drinks/thirst quenchers					

4.7 Do you usually drink fizzy drinks / cola?

With sugar	Without sugar

4.8 To what degree have you changed your intake of fizzy drinks in the last 3 months?

Not at all	Some	A lot

4.9 How many cups of coffee or tea do you drink daily?
(Write 0 if you do not drink coffee or tea daily)

Number cups coffee	
Number cups tea	

4.10 Do you normally salt your food?

Yes, a lot	Yes, some	No

4.11 How often have you consumed alcohol in the course of the past year?
(Low alcohol beer and non-alcoholic beer are not included)

4-7 times/ week	2-3 times /week	Once /week	2-3 times/ month	Once/ month	Few times /year	None past year	Never had alcohol

4.12 When you drink, do you usually drink: (Check more than one if applicable)

Beer	Wine	Spirits

4.13 To what degree have you changed your intake of alcohol in the last 3 months?

Not at all	Some	A lot

4.14 If you have changed your diet the last three months, how has it changed? (Check one or more)

Less fat	
More fat	
Less carbohydrates	
More carbohydrates	
More fruit and vegetables	
Less salt	
More fatty fish	
I have not made any changes	
Other	

5. Tobacco

5.1 Have you smoked/do you smoke daily?

Yes, currently	Yes, previously	Never

5.2 If you smoke daily now, or have smoked before

How many cigarettes do you or did you usually smoke daily?	
How many years altogether have you smoked?	

5.3 Have you used snus/do you use it daily?

Yes, currently	Yes, previously	Never

5.4 If you use snus daily now, or have used it previously:

How many snus do you or did you usually use daily?	
How many years altogether have you used snus?	

6. Education and work

6.1 How many years of schooling/education have you completed altogether?

	Years

6.2 What is your highest achieved education? (Check only one)

Primary and secondary school	
Upper secondary school	
College, 1 year	
College/university, 3 years (Bachelor)	
College/university, 5 years (Master)	
College/university, > 5 years	
PhD	

6.3 Are you currently employed?

Yes, full time	Yes, part time	No	Student

7. Physical activity

7.1 What kind of physical activity have you undertaken in you spare time in the course of the past year?
Estimate a weekly average for the year. From home to work is regarded as spare time. Answer both questions.

	Hours per week			
	None	Less than 1	1-2	3 or more
Light exercise You do not sweat or feel out of breath				
Hard physical activity You sweat and feel out of breath				

7.2 Describe the extent of movement and bodily exertion in your spare time. If the activity varies considerably, e.g. between summer and winter, then give an average. The question applies to the past year only.
(Check the appropriate answer)

Read, watch TV or other sedentary activity?	
Walk, cycle or move about in some other way at least 4 times/week (This should include walking or cycling to work, Sunday stroll/walk, etc.)	
Take part in physical exercise/sport, do heavy gardening work? (Note that the activity must take place at least 4 times a week)	
Exercise hard or take part in competitive sport regularly and several times a week	

8. Use of medicines

8.1 Do you take any of these medicines?

	Currently	Earlier	Never
Medicine for high blood pressure			
Cholesterol-reducing medicine			

8.2 If you have used any of the medicines in 8.1 in the last 4 weeks, give the name and reason for using them:

		How long have you used this medicine?	
Name of medicine	Reason for use	Up to 1 year	More than 1 year

9. Questions for women

9.1 Are you currently pregnant?

Yes	No	Not sure	Past fertile age

9.2 If you use the p-pill, mini-pill, p-injection, hormone loop or oestrogen;
which preparation do you use?

10. Other

Gender	
Age	
To be filled out by health personnel at inclusion in the trial (week 1)	
Height	
Weight	
Waist circumference	
Blood pressure	
Blood glucose	
Blood sample ID	
ID number	

Supplementary material 2

Supplementary Table 1. Paired samples t-test stratified by negative metabolic syndrome diagnosis (MetS-no) in each group and the whole study population at baseline.*

		Norvegia® (n = 32)			Gamalost® (n = 38)			Control (n = 32)			Study population (n = 102)		
Variable		Mean	95% CI	*p*	Mean	95% CI	*p*	Mean	95% CI	*p*	Mean	95% CI	*p*
Waist circumference	Baseline	78.7			78.4			79.8			78.9		
	Change	-0.8	-1.3, -0.3	0.004	-1.5	-2.1, -0.9	<0.001	-1.6	-2.1, -1.1	<0.001	-1.3	-1.6, -1.0	<0.001
Blood glucose	Baseline	5.63			5.45			5.67			5.57		
	Change	0.16	-0.10, 0.41	0.219	0.27	0.05, 0.49	0.016	0.09	-0.19, 0.37	0.510	0.18	0.04, 0.32	0.012
Systolic BP	Baseline	126.1			126.1			127.7			126.6		
	Change	-4.3	-6.7, -2.0	0.001	-3.1	-5.5, 0.7	0.014	-2.9	-6.0, 0.2	0.065	-3.4	-4.9, -2.0	<0.001
Diastolic BP	Baseline	77.9			79.9			79.2			79.1		
	Change	-0.3	-2.2, 1.6	0.773	-2.7	-4.2, -1.1	0.001	-1.0	-3.2, 1.2	0.372	-1.4	-2.4, -0.3	0.011
HDL-cholesterol	Baseline	1.69			1.72			1.75			1.72		
	Change	-0.04	-0.13, 0.05	0.345	-0.06	-0.11, -0.004	0.035	-0.03	-0.10, 0.04	0.362	-0.04	-0.08, -0.004	0.028
Total cholesterol	Baseline	4.95			4.87			5.31			5.04		
	Change	-0.06	-0.24, 0.12	0.508	-0.11	-0.27, 0.05	0.187	-0.15	-0.32, 0.02	0.077	-0.11	-0.20, -0.01	0.029

* Only factors with significant associations are shown.

Supplementary table 2. Paired samples t-test stratified by the absence of each metabolic syndrome factor in each group and the whole study population at baseline.*

		Norvegia®			Gamalost®			Control			Study population		
Variable		Mean	95% CI	*p*	Mean	95% CI	*p*	Mean	95% CI	*p*	Mean	95% CI	*p*
Waist circumference	n	33			36			30			99		
	Baseline	78.0			76.3			77.5			77.2		
	Change	-1.0	-1.6, -0.4	0.001	-1.3	-1.9, -0.7	<0.001	-1.4	-1.9, -0.9	<0.001	-1.2	-1.6, -0.9	<0.001
Triglycerides	n	41			46			42			129		
	Baseline	0.92			0.89			1.01			0.94		
	Change	0.01	-0.09, 0.11	0.816	0.09	0.00, 0.17	0.049	0.22	-0.02, 0.46	0.66	0.11	0.02, 0.20	0.018
Blood glucose	n	18			26			30			62		
	Baseline	5.18			5.08			5.31			5.18		
	Change	0.51	0.16, 0.86	0.007	0.47	0.22, 0.72	0.001	0.27	0.052, 0.48	0.018	0.42	0.27, 0.57	<0.001
Systolic BP	n	23			26			20			69		
	Baseline	119.3			118.6			120.0			119.2		
	Change	-3.6	-7.1, -0.1	0.046	-0.6	-3.3, 2.1	0.641	-0.3	-3.9, 3.4	0.886	-1.5	-3.3, 0.3	0.103
HDL-cholesterol	n	46			48			44			138		
	Baseline	1.70			1.73			1.69			1.70		
	Change	-0.04	-0.11, 0.04	0.314	-0.06	-0.10, -0.01	0.016	-0.05	-0.10, 0.01	0.106	-0.05	-0.08, -0.01	0.006

* Only factors with significant associations are shown.