Effect on bone anabolic markers of daily cheese intake with and without vitamin K₂: a randomised clinical trial

Helge Einar Lundberg, Morten Glasø, Rahul Chhura, Arjun Andre Shukla, Torunn Austlid, Zohaib Sarwar, Kathrine Hovland, Sapna Iqbal, Hans Erik Fagertun, Helge Holo, Stig Einride Larsen

ABSTRACT

Background Daily intake of 57 g Jarlsberg cheese has been shown to increase the total serum osteocalcin (tOC). Is this a general cheese effect or specific for Jarlsberg containing vitamin K₂ and 1,4-dihydroxy-naphtoic acid (DHNA)?

Methods 66 healthy female volunteers (HV) were recruited. By skewed randomisation (3:2), 41 HV were allocated to daily intake of 57 g Jarlsberg (J-group) and 25–50 g Camembert (C-group) in 6 weeks. After 6 weeks the C-group was switched to Jarlsberg. The study duration was 12 weeks with clinical investigations every 6 weeks. The main variables were procollagen type 1 N-telopeptide (PINP), tOC, carboxylated osteocalcin (cOC) and the osteocalcin ratio (Rₜ) defined as the ratio between cOC and undercarboxylated osteocalcin (uOC). Serum cross-linked C-telopeptide type I collagen (CTX), vitamin K₂, lipids and clinical chemistry were used as secondary variables.

Results PINP, tOC, cOC, Rₜ and vitamin K₂ increased significantly (p<0.01) after 6 weeks in the J-group. PINP remained unchanged in the C-group. The other variables decreased slightly in the C-group but increased significantly (p<0.05) after switching to Jarlsberg. No CTX-changes detected in neither of the groups. Serum lipids increased slightly in both groups. Switching to Jarlsberg, total cholesterol and low-density lipoprotein-cholesterol were significantly reduced (p<0.05). Glycated haemoglobin (HbA1c), Ca²⁺ and Mg²⁺ were significantly reduced in the J-group, but unchanged in the C-group. Switching to Jarlsberg, HbA1c and Ca²⁺ decreased significantly.

Conclusion The effect of daily Jarlsberg intake on increased s-osteocalcin level is not a general cheese effect. Jarlsberg contain vitamin K₂ and DHNA which increases PINP, tOC, cOC and Rₜ and decreases Ca²⁺, Mg²⁺ and HbA1c. These effects reflect increased bone anabolism and a possible reduced risk of adverse metabolic outcomes.

Trial registration number NCT04189796.

INTRODUCTION

Vitamin K is important for bone health, and low intake of vitamin K has been associated with increased risk of fractures. Supplementation with vitamin K₂, vitamin D and calcium is a recommended first-line treatment of osteoporosis. Vitamin K₂ is essential for carboxylation or activation of Glu proteins. Osteocalcin is 1 of the 17 Gla proteins in humans, and carboxylated osteocalcin has a key role in bone formation and maintenance. The ratio between circulating carboxylated and undercarboxylated osteocalcin reflects the extrahepatic vitamin K status.

Vitamin K₂ or menaquinone (MK) has many variants. The short-chained MK-4 can be formed from vitamin K₁ in humans and is found in animal products such as liver. The long-chained vitamin K₂ vitamers like MK-7, MK-8, MK-9 and MK-9(4H) are of bacterial origin, and occur in certain fermented foods such as cheese.

These variants have greater extrahepatic activity than MK-4 and vitamin K₁; possibly due to more efficient uptake and much longer serum half-life. Prospective cohort
studies have demonstrated health benefits that can be attributed to the intake of vitamin \(K_2\), but not \(K_1\). In the Western diet cheese is the major source of long-chained vitamin \(K_2\).\(^5\)

MK-9 is the most important \(K_2\) vitamer in cheese, but the amount varies considerably.\(^6\) Some cheeses also contain MK-9(4H) and Jarlsberg cheese is particularly rich in both MK-9 and MK-9(4H).\(^7\) This cheese is made with lactic acid bacteria producing MK-8 and MK-9 and Propionibacterium freudenreichii producing MK-9(4H).

A previous dose-response study of daily Jarlsberg with healthy premenopausal women was performed with a three-level between patient response surface pathway design.\(^8,9\) The maximum efficacy dose (MED) of Jarlsberg in order to obtain increased vitamin \(K_2\) status, measured as osteocalcin carboxylation and total serum osteocalcin (tOC) level was estimated to 57 g/day.\(^10\) These results were confirmed in a recent clinical study on the same study population.\(^11\) The MED of 57 g/day Jarlsberg cheese was verified to increase the vitamin \(K_2\) status and tOC level.\(^11\) The maintenance dose for this study population was estimated to 45 g/day of Jarlsberg and verified as sufficient to keep the obtained tOC level. Osteocalcin is used as a serum marker of osteoblastic bone formation indicating that Jarlsberg stimulates bone formation.\(^12\) Bone markers like procollagen type 1 N-terminal propeptide (PINP) and serum cross-linked C-telopeptide type I collagen (CTX) were not recorded.\(^13,14\)

Although cheese has been associated with vitamin \(K_2\)-related health benefits, the effect of cheese consumption on bone health has, to the best of our knowledge, never been investigated in human controlled clinical trials. Because of its high vitamin \(K_2\) content, Jarlsberg appears to be a good candidate for such studies.

1,4-dihydroxy-2-naphthoic acid (DHNA) has been shown to have anti-osteoporotic effects and increase bone mineral density in ovariectomised mice.\(^15\) Jarlsberg contains Propionibacterium freudenreichii, which produces MK-9(4H), but also secretes DHNA. This might explain the increase in tOC as well as the carboxylation of osteocalcin (cOC) and it has been suggested that DHNA could be used in treatment of postmenopausal osteoporosis.\(^15\)

If the raise of tOC and cOC levels is not a general cheese effect, but a Jarlsberg effect, further studies on Jarlsberg as prophylaxis are even more relevant. The aim of this study was to compare the effect of daily Jarlsberg cheese intake with a cheese without vitamin \(K_2\), but similar fat and protein content, on PINP, tOC, cOC, \(R_p\) and glucose metabolism by glycated haemoglobin (HbA1c).

**MATERIAL AND METHODS**

**Population and sample**
The study population consisted of healthy Norwegian women (healthy volunteer (HV)) of premenopausal age. Pregnant women and women suffering from known gastrointestinal disorder, abnormal liver or kidney function, lactose intolerance or known milk product allergy, diabetes mellitus or verified cancer were excluded. Women under systemic treatment with corticosteroids or immunosuppressive drugs the last 3 weeks or participating in another clinical trial the last 6 weeks before start of the study were not included.

Sixty-eight HVs were recruited by eight general practitioners from Viken County in Norway. Two HVs dropped out from the study for personal reasons during the first week. The study sample consisted of 66 HVs with a mean age of 33.6 years (range: 19.4–51.9 years) and body mass index of 24.3 kg/m\(^2\) (range: 15.0–35.4). By randomisation, 41 HVs were allocated to daily intake of Jarlsberg cheese (J-group) and 25 to Camembert cheese (C-group). The two groups were found comparable on all recorded demographic data, concomitant diseases and treatment (table 1). All ongoing treatments were kept unchanged during the study. Vitamin D deficiency was initially recorded in nine participants, and supplements were provided to obtain normal range.

**Study design and allocation**
The study was performed as an open, randomised multicentre trial with semi crossover design.\(^16\) The participants were randomised for either 6 weeks of Jarlsberg or 6 weeks of Camembert cheese. The participants allocated to Camembert were then switched to Jarlsberg for an additional 6 weeks. The results obtained during the first 6 weeks and the 6 weeks after switch in cheese are reported. The study subjects were skewed randomly allocated (2:3) in two treatment groups; 41 in the J-group and 27 in the C-group. The allocation of participants to treatment groups was performed by the data manager prior to the start of the study by stratified randomisation with a fixed block size of 10.\(^17\) Two HVs withdrew, reducing the C-group to 25. The participants were asked not to change their usual diet during the study except for avoiding other cheese than the cheese provided in the study. No diet registration was performed, but the participants daily registered their intake of either Jarlsberg or Camembert during the study. The cheese compliance in the study was 95% and 92% in the J-group and the C-group, respectively. The compliance in the C-group after switching to Jarlsberg was 93%.

**Clinical procedure**
The clinical part of the study was performed from March 2020 to August 2020. Clinical status and blood sampling were performed initially and every 6 weeks. In addition to common laboratory variables, the blood samples were used for measurements of osteocalcin; PINP; CTX; lipids and the vitamin \(K_2\) vitamers MK-7, MK-8, MK-9 and MK-9(4H).

**Clinical intervention**
The J-group received 57 g/day or six slices Jarlsberg, and the C-group 50 g/day of TINE Camembert cheese for 6 weeks. Participants of the J-group received 100 g packages containing cheese slices of 10 g. The C-group received
150 g packages, divided in three equal parts. The corresponding energy and fat intake per day is 680 kJ and 14.0 g in the C-group, and 729 kJ and 15.4 g in the J-group. Both the Jarlsberg and Camembert are produced by TINE SA and cheese from three production batches was used.

The average vitamin K per 100 g of Jarlsberg cheese contained 3.0 µg vitamin K1, 5.2 µg MK-4 and 1.5 µg MK-7, 6.7 µg MK-8, 23.9 µg MK-9 and 40.5 µg MK-9(4H). TINE Camembert cheese does not contain vitamin K.

**Vitamin K extraction and osteocalcin analysis**

Vitamin K in cheese and blood samples was analysed as described previously. The cOC and the undercarboxylated osteocalcin (ucOC) were measured in plasma by immunoassays kits (Takara Bio, Ōtsu, Japan) by Vitas AS. The tOC is defined as the sum of cOC and ucOC.

**Central variables**

The three main variables were PINP, tOC and the osteocalcin ratio R_o=ocOC/ ucOC. The supporting variables were sum of vitamin K, and the variants MK-7, MK-8, MK-9 and MK-9(4H). Additionally CTX, Glycated haemoglobin; the lipids; haematological and clinical chemistry were used as secondary variables.

**Randomisation**

The participants were skewed allocated (2:3) to the J-group and C-group by block randomisation with fixed block size of 10.

**Statistical analysis**

A significance level of 0.05; a power of 90% and a clinical relevant difference between groups of one time SD, a minimum of 23 participants are required in each group.
The assumed continuously distributed variables were expressed by mean values with 95% CI. As an index of dispersion, SD were given. Categorised variables were given in contingency tables. All tests were performed two-tailed with a significance level of 0.05. Analysis of covariance with baseline measurement as covariate was used for comparison of groups.

Changes within groups were tested by using a paired two-tailed t-test.

**Approvals**
The study was performed in accordance with the research protocol: ‘Effect of Jarlsberg cheese compared with cheese without vitamin K2 (Camembert) regarding increased Osteocalcin level in healthy women’, (ClinicalTrial.gov).

**RESULTS**
The tOC level increased significantly (p<0.01) from 21.4 ng/mL (95% CI: 16.6 to 26.2) to 25.8 ng/mL (95% CI: 21.0 to 30.6) after 6 weeks in the J-group ([figure 1A](#fig1a)). In the C-group this level was slightly reduced from 23.3 ng/mL (95% CI: 13.7 to 32.8) to 21.3 ng/mL (95% CI: 13.3 to 29.3). The difference in tOC increase between groups was 6.4 ng/mL (95% CI: 13.7 to 32.8) and significantly (p<0.01) in favour of Jarlsberg cheese. After switching to daily intake of Jarlsberg in the C-group, the tOC level increased significantly (p=0.05) from 21.3 ng/mL to 25.1 ng/mL (95% CI: 18.8 to 31.4) ([figure 1A](#fig1a)). A similar pattern was detected for cOC and the Ro ([table 2](#tab2)). Both variables increased significantly in the J-group, but remained unchanged in the C-group. The increase in cOC and Ro was significant different in the two groups (p<0.01; p=0.04). The level of ucOC was nearly unchanged in both groups. After switching to Jarlsberg in the C-group, both the cOC and the Ro increased significantly (p<0.01; p=0.05) whereas the ucOC were unaffected ([table 3](#tab3)).

PINP increased significantly (p<0.01) in the J-group, but remained unchanged in the C-group ([table 2](#tab2)). The mean difference between the groups was 9.4 ng/mL (95% CI: 1.3 to 9.3) and significant (p=0.01) in favour of Jarlsberg, CTX was unaffected in both groups during the study. After switching to Jarlsberg in the C-group, PINP increased, but not significantly ([table 3](#tab3)). However, PINP decreased in 7 of the 25 participants; a proportion significantly (p<0.05) below 50%. Four of these seven HVs had a remarkably large PINP reduction, resulting in the lack of significance on the mean level. CTX was unaffected after switching in cheese.

The sum of vitamin K2 vitamers increased significantly (p<0.01) from 0.38 ng/mL (95% CI: 0.30 to 0.45) to 0.72 ng/mL (95% CI: 0.64 to 0.81) after 6 weeks in the J-group ([figure 1A](#fig1a)). A significant reduction (p=0.04) was detected in the C-group. The development in sum of vitamin K2 was significant in favour of the J-group (p<0.01). After switching to Jarlsberg in the C-group, the sum of vitamin K2 increased significantly (p<0.01) from 0.41 ng/mL (95% CI: 0.34 to 0.48) to 0.65 ng/mL (95% CI: 0.53 to 0.79) ([figure 1B](#fig1b)). All the vitamin K2 vitamers MK-7, MK-8, MK-9 and MK-9(4H) increased significantly (p<0.01) after 6 weeks in the J-group but decreased in the C-group (0.02≤p≤0.21) ([table 2](#tab2)). The increases in all MK-variants were significant in favour of Jarlsberg cheese. After switching from Camembert to Jarlsberg, MK-8, MK-9 and MK-9(4H) increased significantly (p<0.01) whereas MK-7 was nearly unchanged ([table 3](#tab3)).

Mean HbA1c decreased significantly (p<0.01) after 6 weeks in the J-group ([table 4](#tab4)). In the C-group this level was significantly increased (p=0.05) The mean difference in HbA1c development between groups was significantly (p<0.01) in favour of Jarlsberg cheese. After switching to daily intake of Jarlsberg in the C-group, mean HbA1c decreased significantly (p<0.01) ([table 5](#tab5)).

Triglycerides, low-density lipoprotein (LDL)-cholesterol, total cholesterol and the cholesterol ratio...
LDL/high-density lipoprotein (HDL) increased slightly, but significantly (p≤0.05) in both groups during the first 6 weeks of cheese intake (table 4). No significant difference was detected between groups. After switching cheese in the C-group, total cholesterol and LDL-cholesterol were significantly reduced (p≤0.05). A slight but not significant reduction was detected in the triglycerides and the cholesterol ratio (table 5).

Ca ++ and Mg ++ were significantly reduced in the J-group (p≤0.05) but remained unchanged in the C-group (table 4). The differences between the groups were found significant (p≤0.05). After switching cheese in the C-group, Ca ++ were significantly reduced (p≤0.05), but Mg ++ was found unchanged (table 5).

Phosphate increased in the J-group, but reduced in the C-group. None of the changes were significant, but the difference between groups was borderline significant (table 4). When switching to Jarlsberg in the C-group, the phosphate increased, but not significantly (table 5).

Urea increased significantly (p<0.01) in the J-group and decreased non-significantly in the C-group (table 4). The difference between groups was found significant (p<0.01). No change in urea was detected in the C-group after the switch to Jarlsberg (table 5). Creatinine was reduced significantly (p<0.01) in the C-group, but non-significantly in the J-group (table 4). The difference between groups was not significant. No change in creatinine was detected in the C-group after the switch to Jarlsberg cheese (table 5).

**DISCUSSION**

In the J-group, the tOC and cOC increased significantly confirming the results in the two previous dose-response-studies. In the C-group the tOC was slightly, but not significantly reduced after the first 6 weeks. Switching from Camembert cheese to Jarlsberg was led to a significant increase in tOC and cOC. This supports that Jarlsberg has a significant stimulatory effect on serum osteocalcin. A Mediterranean diet enriched with virgin olive oil has been associated with increased tOC in elderly men. The present study is probably the first time dietary intervention has been able to demonstrate a significant increase in the tOC level in a randomised controlled trial.

Measurable changes in Bone Mass Density (BMD) require a minimum of 1 year observation. Like tOC,

**Table 2** Comparison of Jarlsberg and Camembert cheese in development of osteocalcin variables and bone markers and the vitamin K₂ vitamers during 6 weeks of daily cheese intake. The results are expressed by mean values with SD in bracket and 95% CIs

<table>
<thead>
<tr>
<th>Variables</th>
<th>Baseline</th>
<th>Camembert</th>
<th>Week 6</th>
<th>Camembert</th>
<th>Week 6—baseline</th>
<th>P values between groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>cOC=carboxylated osteocalcin (ng/mL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jarlsberg (n=41)</td>
<td>11.2 (9.0)</td>
<td>11.7 (9.6)</td>
<td>11.2 (9.0)</td>
<td>11.5 (9.4)</td>
<td>11.3 (9.6)</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>Camembert (n=41)</td>
<td>15.3 (9.5)</td>
<td>11.2 (9.4)</td>
<td>11.7 (9.6)</td>
<td>12.3 (9.7)</td>
<td>11.9 (9.6)</td>
<td></td>
</tr>
<tr>
<td>Phosphate increased (mg/L)</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Jarlsberg (n=41)</td>
<td>8.4 to 14.1</td>
<td>8.3 to 14.1</td>
<td>8.2 to 13.7</td>
<td>8.2 to 13.3</td>
<td>8.0 to 13.4</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>Camembert (n=41)</td>
<td>12.3 to 18.3</td>
<td>12.3 to 18.3</td>
<td>12.3 to 18.3</td>
<td>12.3 to 18.3</td>
<td>12.3 to 18.3</td>
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</tr>
<tr>
<td>ucOC=undercarboxylated osteocalcin (ng/mL)</td>
<td></td>
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</tr>
<tr>
<td>Jarlsberg (n=41)</td>
<td>10.2 (10.4)</td>
<td>10.5 (10.5)</td>
<td>10.5 (10.5)</td>
<td>10.5 (10.5)</td>
<td>10.5 (10.5)</td>
<td></td>
</tr>
<tr>
<td>Camembert (n=41)</td>
<td>6.9 to 13.5</td>
<td>6.3 to 14.4</td>
<td>6.5 to 8.6</td>
<td>6.5 to 8.6</td>
<td>6.5 to 8.6</td>
<td>p=0.14</td>
</tr>
<tr>
<td>PINP=procollagen (ng/mL)</td>
<td>61.1 (27.5)</td>
<td>61.4 (24.1)*</td>
<td>69.1 (21.7)</td>
<td>58.7 (21.7)</td>
<td>10.4 (24.1)*</td>
<td>p=0.01</td>
</tr>
<tr>
<td>CTX=collagen I (ng/mL)</td>
<td>0.29 (0.13)</td>
<td>0.27 (0.17)*</td>
<td>0.27 (0.15)*</td>
<td>0.27 (0.15)*</td>
<td>0.28 (0.15)*</td>
<td></td>
</tr>
<tr>
<td>MK-7 (ng/mL)</td>
<td>0.12 (0.07)</td>
<td>0.21 (0.17)</td>
<td>0.21 (0.17)</td>
<td>0.21 (0.17)</td>
<td>0.30 (0.30)</td>
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<tr>
<td>MK-8 (ng/mL)</td>
<td>0.09 (0.07)</td>
<td>0.15 (0.07)</td>
<td>0.15 (0.07)</td>
<td>0.15 (0.07)</td>
<td>0.15 (0.07)</td>
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<tr>
<td>MK-9 (ng/mL)</td>
<td>0.09 (0.09)</td>
<td>0.11 (0.07)</td>
<td>0.11 (0.07)</td>
<td>0.11 (0.07)</td>
<td>0.11 (0.07)</td>
<td></td>
</tr>
<tr>
<td>MK-9(4H) (ng/mL)</td>
<td>0.08 (0.06)</td>
<td>0.10 (0.07)</td>
<td>0.10 (0.07)</td>
<td>0.10 (0.07)</td>
<td>0.10 (0.07)</td>
<td></td>
</tr>
<tr>
<td>R₀ = carboxylated/undercarboxylated</td>
<td>1.47 (0.94)</td>
<td>1.86 (1.14)</td>
<td>2.19 (1.3)</td>
<td>2.09 (1.34)</td>
<td>0.44 (1.01)</td>
<td>p=0.03</td>
</tr>
<tr>
<td>Ro, osteocalcin ratio; ucOC, undercarboxylated osteocalcin.</td>
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</tbody>
</table>

*Two baseline blood samples in the Camembert group were lost and reduced the sample to 23 for estimation and comparison of the development in PINP and CTX.

cOC, carboxylation osteocalcin; CTX, C-telopeptide type I collagen; MK, menaquinone; PINP, procollagen type 1 N-terminal propeptide; R₀, osteocalcin ratio; ucOC, undercarboxylated osteocalcin.
PINP is an osteoblast-derived bone formation marker.\textsuperscript{13} CTX is a marker of osteoclast activity and used to assess the level of bone resorption.\textsuperscript{14} PINP and CTX are used as bone turnover biomarkers and may predict changes in BMD within 1–3 months.\textsuperscript{22} The present study included variables is significant, but substantially weaker than the BMD within 1–3 months.\textsuperscript{22} The present study included as bone turnover biomarkers and may predict changes in the level of bone resorption.\textsuperscript{14} PINP and CTX are used to assess CTX is a marker of osteoclast activity and used to assess increase BMD. The decreases in Ca\textsuperscript{2+} and Mg\textsuperscript{2+} caused by consumption of Jarlsberg probably also reflect uptake from the blood for net bone formation. Daily intake of Camembert did not show these effects.

The vitamin K\textsubscript{2} vitamers MK-7, MK-8, MK-9 and MK-9(4H) all increased significantly during the 6 weeks in the Jarlsberg group. Significant reductions were recorded in the C-group. After switching to Jarlsberg, the increase in vitamin K\textsubscript{2} in the C-group showed an almost parallel pattern to the increase in the J-group. There was a high degree of covariation between the rise of tOC and vitamin K\textsubscript{2}. The calculated correlation between these two variables is significant, but substantially weaker than the graphical illustration may indicate, and was obvious in the previous studies.\textsuperscript{10,11} In the dose-response study, the level of vitamin K\textsubscript{2} increased with increasing cheese dose whereas tOC and cOC showed a maximum at 57 g cheese a day and falling trends above this dose.\textsuperscript{10} A similar pattern was also detected in the dose de-escalation study.\textsuperscript{11} This should not be the case if vitamin K\textsubscript{2} was the only factor which increases tOC. Thus the increase in tOC cannot solely be ascribed to the vitamin K\textsubscript{2} content of Jarlsberg.

In vitro, vitamin K\textsubscript{2} has been shown to stimulate osteoblast differentiation and mineralisation, and some human studies have found a BMD increase in postmenopausal women taking 45 mg daily of vitamin K\textsubscript{2}.\textsuperscript{23,24} The vitamin K\textsubscript{2} in Jarlsberg is formed by lactic acid bacteria and propionic acid bacteria. These vitamin K\textsubscript{2} vitamers are different from those K\textsubscript{2}-tablets tested in clinical trials. Propionibacterium freudenreichii has been found to produce DHNA; an intermediate in the vitamin K\textsubscript{2} biosynthesis.\textsuperscript{25} It has been suggested that DHNA could be an alternative or at least an additional treatment of postmenopausal osteoporosis.\textsuperscript{16} In ovariectomised mice, this compound was shown to stimulate the production of osteocalcin, while vitamin K\textsubscript{2} could not.\textsuperscript{16} DHNA is the possible key to the tOC stimulatory effect of Jarlsberg. DHNA is formed as an intermediate in the biosynthesis of vitamin K2 in all organisms that produce the vitamin. However, to our knowledge propionic acid bacteria are the only bacteria reported to produce DHNA in substantial excess.

These results may come out additionally as important facts for future pharmaceutical interventions.

The participants were asked to maintain their usual diet during the study except for any cheese provided in the study. The cheese compliances were found satisfactory.
but the daily diet or possible change in diet was not recorded. This might reduce the value of the obtained results to some extent. However, the participants were properly followed-up during the study and a systematic significant change in the diet it is not likely.

Some studies on Asian women have reported that high doses of vitamin K₂ gave an increase in BMD, but this has not been detected in Europeans. The bone anabolic effects of daily Jarlsberg intake is more possibly caused by its content of DHNA with stronger osteoblast stimulation compared with vitamin K₂. A possible explanation is that the increased tOC is mainly caused by DHNA and carboxylated by the long-chained vitamin K₂ in Jarlsberg. In this process, calcium and additionally magnesium is absorbed from serum and increases BMD. Further studies should be performed to investigate if daily intake of Jarlsberg cheese could affect bone mass and structure in humans. Such studies could be performed on borderline, untreated osteoporotic patients or on endurance athletes. Several of such athletes suffer from decreased BMD due to energy deficiency; reduced resistance to infections and lack of nutritional factors.

During the 6 weeks daily intake of 57 g Jarlsberg, the mean HbA₁c was significantly reduced with 3% and increased with 2% in the C-group. Switching from Camembert cheese to Jarlsberg led to a significant 3% decrease in HbA₁c. The inverse association of tOC with blood glucose, HbA₁c, the risk of type 2 diabetes and metabolic syndrome is previously reported. The mechanisms underlying these effects in humans are unknown, but the role of osteocalcin as a hormone controlling energy metabolism has been suggested. The obtained reduction of HbA₁c during daily Jarlsberg intake indicates that these associations are maintained even in the healthy population, and suggests that daily intake of Jarlsberg can be an aid in controlling major lifestyle-related diseases.

In the previous dose-response study of Jarlsberg a significant reduction of blood lipids was observed. All the results obtained in this dose-response study were verified in the de-escalation study except for the reduction in blood lipids. This may possibly be due to temporary

<table>
<thead>
<tr>
<th>Table 4</th>
<th>Comparison of Jarlsberg and Camembert cheese in development of biochemical variables during 6 weeks of daily cheese intake. The results are expressed by mean values with SD in bracket and 95% CIs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variables</td>
<td>Baseline Jarlsberg (n=41)</td>
</tr>
<tr>
<td>Triglyceride (mmol/L)</td>
<td>1.0 (0.6)</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/L)</td>
<td>2.7 (0.7)</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/L)</td>
<td>1.7 (0.4)</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>4.6 (0.7)</td>
</tr>
<tr>
<td>LDL/HDL</td>
<td>1.7 (0.6)</td>
</tr>
<tr>
<td>Creatinine (µmol/L)</td>
<td>60.9 (10.0)</td>
</tr>
<tr>
<td>Urea (µmol/L)</td>
<td>5.15 (1.25)</td>
</tr>
<tr>
<td>Glycated haemoglobin (mmol/L)</td>
<td>34.2 (2.8)</td>
</tr>
<tr>
<td>Calcium (Ca+1) (mmol/L)</td>
<td>2.34 (0.09)</td>
</tr>
<tr>
<td>Magnesium (Mg+2) (mmol/L)</td>
<td>0.86 (0.06)</td>
</tr>
<tr>
<td>Phosphate (mmol/L)</td>
<td>1.20 (0.16)</td>
</tr>
</tbody>
</table>

HDL, high-density lipoprotein; LDL, low-density lipoprotein.
changes in dietary habits and physical activity caused by measures to limit the spread of SARS-CoV2. In the present study, such factors might explain the lack of blood lipid reduction during the first 6 weeks. After switching to Jarlsberg in the C-group, a significant reduction of the lipids was observed. This is in line with the inverse association between tOC and body fat reported in prospective studies. The present study detected an increase in s-phosphate connected with daily Jarlsberg intake which might indicate that this cheese is not suitable for nutrition in end stage renal disease.

CONCLUSION
The effect of daily Jarlsberg intake on increased s-ostecalcin level is not a general cheese effect. Jarlsberg containing vitamin K2 and DHNA increases tOC, cOC, RO and PINP and decreases s-Ca++, s-Mg++ and HbA1c. These effects reflect increased bone anabolism and a possible reduced risk of adverse metabolic outcomes.

Author affiliations
1Skjetten Medical Center, Skjetten, Norway
2Stallbakken Medical Center, Rælingen, Norway
3Sundet Medical Center, Eidsvoll, Norway
4Meddoc Research, Skjetten, Norway
5Norwegian University of Life Sciences, As, Norway
6Clinical Department, Meddoc Research, Lillestrøm, Skjetten, Norway
7Veterinary Medicine, Norwegian University of Life Sciences, As, Oslo, Norway

Contributors All the authors participated in construction of the trial protocol, manuscript writing and approved the manuscript. The author acting as guarantor is HEL. TINE SA provided Jarlsberg and Camembert cheese, along with financial support, but did not play any role in the design, implementation, analysis, interpretation or manuscript writing. MSc Vivy Liang Larsen, Meddoc was in charge of Data Management and BSc Natharat Thendiokkul, Meddoc in charge of all clinical monitoring. Vitas Laboratory performed all the osteocalcin analyses.

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Ethics approval This study involves human participants and was approved by Norwegian Regional Ethical Committee South-East, project nr.84845. Participants gave informed consent to participate in the study before taking part.

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Data availability statement Data are available upon reasonable request. All data is saved in a SAS database at Meddoc Research.

Table 5 Development in lipid and biochemical variables after switching from Camembert to 6 weeks daily intake of Jarlsberg cheese. The results are expressed by mean values with SD in bracket and 95% CIs

<table>
<thead>
<tr>
<th>Variables</th>
<th>Baseline Jarlsberg (n=25)</th>
<th>6 weeks on Jarlsberg (n=25)</th>
<th>Week 6—baseline (n=25)</th>
<th>P values within variables</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglyceride (mmol/L)</td>
<td>1.4 (0.8)</td>
<td>1.3 (0.6)</td>
<td>−0.1 (0.7)</td>
<td>p=0.32</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/L)</td>
<td>3.4 (0.9)</td>
<td>3.1 (0.8)</td>
<td>−0.3 (0.5)</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/L)</td>
<td>1.6 (0.3)</td>
<td>1.5 (0.3)</td>
<td>−0.1 (0.2)</td>
<td>p=0.05</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>5.1 (0.7)</td>
<td>4.7 (0.6)</td>
<td>−0.4 (0.4)</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>LDL/HDL cholesterol</td>
<td>2.3 (0.9)</td>
<td>2.2 (0.9)</td>
<td>−0.1 (0.3)</td>
<td>p=0.20</td>
</tr>
<tr>
<td>Creatinine (µmol/L)</td>
<td>61.2 (8.9)</td>
<td>61.3 (6.2)</td>
<td>0.2 (6.4)</td>
<td>p=0.90</td>
</tr>
<tr>
<td>Urea (mmol/L)</td>
<td>5.39 (1.62)</td>
<td>5.42 (1.63)</td>
<td>0.0 (1.5)</td>
<td>p=0.92</td>
</tr>
<tr>
<td>Glycated haemoglobin (mmol/L)</td>
<td>33.8 (3.1)</td>
<td>32.8 (2.4)</td>
<td>−1.0 (1.2)</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>Calcium (Ca++) (mmol/L)</td>
<td>3.26 to 35.1</td>
<td>31.9 to 33.8</td>
<td>−1.5 to −0.5</td>
<td>p=0.03</td>
</tr>
<tr>
<td>Magnesium (Mg++) (mmol/L)</td>
<td>0.86 to 0.87</td>
<td>0.83 to 0.88</td>
<td>−0.02 to 0.03</td>
<td>p=0.72</td>
</tr>
<tr>
<td>Phosphate (mmol/L)</td>
<td>1.10 to 1.22</td>
<td>1.12 to 1.28</td>
<td>−0.02 to 0.10</td>
<td>p=0.19</td>
</tr>
</tbody>
</table>

HDL, high-density lipoprotein; LDL, low-density lipoprotein.
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ORCID ID
Helge Einar Lundberg http://orcid.org/0000-0003-3430-9668

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