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Original Research Article

Cheese positively affects serum osteocalcin levels, bone turnover markers and bone mineral density in cross-country skiers: a dose-response study

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ABSTRACT

Background: Vitamin K_2-rich Jarlsberg Cheese is demonstrated to increase Osteocalcin and bone turnover markers in fertile women. The impact on endurance-trained young cross-country skiers (XCS) with elevated bone turnover remains unclear. Purpose of study was to estimate the optimal daily efficacy dose of Jarlsberg cheese to increase serum Osteocalcin level in young female and male XCS and estimate the Jarlsberg effect on bone turnover markers (BTMs) and bone mineral density (BMD).
Methods: In a parallel group study consisting of three design levels, 10 female and 10 male XCS were included, using Response Surface Pathway design. Blood samples were taken at each level for measurements of Osteocalcin, vitamin K_2, BTMs and other biochemical parameters. Resting metabolic rate (RMR), BMD, VO_2max and muscle strength were measured at start and at the end of the study.
Results: The Osteocalcin development with increasing dose of Jarlsberg cheese was almost parallel in both sexes. These variables were reduced significantly from baseline during the first two design levels but increased above baseline by the end of the study. BTMs decreased significantly during the first and second level but increased during the third level. Total and L1-L4 BMD, s-phosphate, s-urea, RMR, muscle strength and Peak VO_2 increased significantly while s-calcium and s-magnesium decreased.
Conclusions: Estimated OED of Jarlsberg cheese was 73 and 84 g/day for females and male athletes, respectively. The development in OC, BTMs and BMD suggest an antiresorptive and perhaps anabolic effect of Jarlsberg Cheese on bone tissue. VO_2 max, RMR and muscular strength development indicated an anabolic situation.

Keywords: Athletes, BMD, BTMs, Jarlsberg Cheese, Osteocalcin, Vitamin K_2

INTRODUCTION

It is well established that dietary calcium and vitamin D are beneficial for skeletal health. Additionally, it has been documented with increased evidence, similar valuable effect from vitamin K_2.[1] Many dairy products are good calcium sources, and some cheeses are also important sources of vitamin K_2. This indicates that cheese

consumption may strengthen bones, but the effect of eating cheese specifically rich in vitamin K_2 has not been sufficiently documented. Vitamin K_2 has been approved as an aid for treating osteoporosis in Japan, but the recommended dose is high.[2] Vitamin K_2 is essential in the carboxylation process activating osteocalcin (OC), that has a key role in bone-formation.[3-5] OC is important for building strong bones, and has a beneficial effect on vascular health, lipid pattern and obesity.[6] Moreover, OC has been reported to improve muscular strength in mice, but this is debated.[7] In postmenopausal osteoporotic women serum OC level has been found to correlate positively with skeletal muscle mass.[8],The long-chained K_2 vitamers like MK-9 (4H) have proven to be much more efficient in activating OC than short chained K_2 and K_1. In the western diet, fermented dairy products such as cheese are the best sources of long-chain vitamin K_2, and benefits have been reported for skeletal and cardiovascular health from intake of vitamin K_2-rich cheese.[3,4,8,9] The dominating K_2 vitamer in most cheeses is MK-9, but the amount varies considerably.[10] Some cheeses also contain MK-9 (4H). Jarlsberg cheese is rich in this vitamer.[11] Jarlsberg cheese is made by milk fermentation with lactic acid bacteria producing MK-8 and MK-9 and *Propionibacterium freudenreichii* (PF) producing MK-9 (4H) and additionally 1,4-dihydroxy-naphthoic acid (DHNA). DHNA has been shown to increase the OC level and have anti-osteoporotic effect in ovariectomized mice by increasing bone density and trabecular thickness.[12] Our previously published dose-response study estimating an optimal daily Jarlsberg dose (OED) for OC carboxylation in healthy fertile women, revealed that the carboxylated osteocalcin (cOC) and the OC ratio increased significantly with increasing Jarlsberg dose up to 57 g/day.[13] These results were verified in a follow up study estimating a maintenance daily dose of Jarlsberg.[14]

Finding increased bone turnover markers (BTMs) after intake of Jarlsberg Cheese in our comparison-study indicates a reduced risk for bone loss.[15] A dose response study on athletes specifically, should be performed because their dietary demands caused by higher level of energy expenditure, and muscular and skeletal repair differ markedly from the previous Jarlsberg study population. This may be especially important for athletes in weight-sensitive sports, for which measures are needed to reduce the health burden associated with problematic low energy availability, including high frequency of low bone mineral density (BMD).[16] Cross-country Skiers (XCS), representing a weight sensitive endurance sport, are at high risk of being exposed to low energy availability (LEA), and they are a suitable group for studying the effects of Jarlsberg cheese.[13,16] With respect to previous studies it was of interest to follow the development in muscle strength and VO_2max with a view to planning a controlled randomised study with the OED.[7,8] The aim of this study was to estimate OED of Jarlsberg cheese with respect to total osteocalcin (tOC) and cOC level in young, female, and male XCS. Further, it was of interest to study the effect of daily Jarlsberg cheese intake on BTMs, bone mineral density (BMD), muscle strength Resting Metabolic Rate (RMR) and VO_2 max in the same sample.

METHODS

Study population

The study population consisted of male and female XCS at a highly competitive level, within tier 3-4, and chosen as a representative group of endurance athletes representing a weight sensitive sport.[17] Exclusion criteria were clinical eating disorders, known gastrointestinal disorder, abnormal liver or kidney function, lactose intolerance or known milk product allergy, diabetes mellitus or verified cancer. So were systemic treatment with corticosteroids or immunosuppressive drugs the last three weeks or participating in another clinical trial the last six weeks before start of the study. After reading the information the recruited XCS signed the informed consent form, an fulfilled all the inclusion- and none of the exclusion criteria.

Study sample size

The study sample consisted of ten female and ten male XCS and were recruited by the primary investigator from ski-teams in Oslo and Viken county from 2 January 2021 to 10 April 2021. The mean age of the study sample was 19.7 years (Range: 18-25 years) with a mean Body Mass Index (BMI) of 22.03 kg/m (Range: 18.3-24.3). All ongoing treatments were kept unchanged during the study. The study period started on 5 May 2021after ending the competitive season and ended on 12 August 2021, before the hardest training period.

Study design

Each of the two parallel sex arms in the study was performed as an open single centre one-dimensional study using within-patient Response Surface Pathway (RSP) design with skewed starting point and stochastic cheese dose-window.[18] The study consisted of three design levels, each of four weeks duration.

In our previous studies, the initial cheese dose window was (20-100) g/day and by using the common mid-point strategy the suggested starting dose was 60 g/day.[13] The dose-curve on the total OC is shown to be a quadratic function in Jarlsberg dose.[13] To be sure of starting below the OEDs, the initial dose was set to m=47 g/day in both sex arms. This gives an adjusted starting dose window of (20-74) g/day.[18] The individual result in each design level recommends the dose in the next. In case the results for a given participant in the first design level reporting a positive cOC increase <10%, the RSP-procedure recommend a maximum dose escalation in the second design level. If this is the results from all the XCS in the given sex arm, the new dose-window will then be

changed with the previously used starting dose as lower boundary. The dose-window for the second design level would then be changed to (47-85) g/day with common cheese dose of 66 g/day and similar corrections in the RSP procedure.[18]

Investigational product

Jarlsberg cheese is produced by TINE SA. Cheese from three Norwegian batches was used in this study. Jarlsberg cheese was delivered in 250 gram per package containing 16 slices. The weight of each slice varies from 14.7 to 16.6 gram with a mean of 15.6 gram. The energy of 100gram Jarlsberg cheese is 351 kcal, originating from 27gram fat, 27gram protein and no carbohydrates. 100 g of Jarlsberg cheese contained on average 3.0µg vitamin K_1, 5.2 µg MK-4, 1.5 µg MK-7, 6.7 µg MK-8, 23.9 µg MK-9 and 40.5 µg MK-9 (4H).

Clinical procedure

Clinical status and blood sampling were performed initially and every four weeks. In addition to common laboratory variables like haematology, electrolytes and sex hormones, the blood samples were used for measurements of OC, procollagen type 1 N-terminal propeptide (PINP), serum cross-linked C-telopeptide type I collagen (CTX), bone specific alkaline phosphates (BAP) and the K_2 vitamers MK-7, MK-8, MK-9, and MK-9 (4H). Vitamin D-analyses were part of the baseline blood analyses. All participants were provided 40 µg Vitamin D supplement/day as a countermeasure taken to control for effects of sun exposure during the summer months in Scandinavia, and to nullify the differences among the participants in Vitamin D-levels after the winter. The XCSs were asked not to change their usual diet, but to replace other cheeses with the Jarlsberg cheese used in the study. They were asked to register their cheese intake in a cheese- registration form collected by the investigator every fourth week during the study. Ahead of the study the participants were asked to register all dietary intake for four days in an electronic application (APP) based on the Norwegian Food Composition Table.[19] In this APP the athletes were not able to see their calorie or nutrient intake. BMD, muscle strength, VO_2 max and resting metabolic rate (RMR) were measured at baseline and at the end of the study.

Variables

in adition to tOC, the primary variables were cOC, undercarboxylated OC (ucOC) and the OC ratio, R_o=cOC/ucOC. The supporting variables were the sum of Vitamin K_2 and the individual MK-vitamers, CTX, PINP and BAP. Secondary variables were BMD, RMR and RMR-ratio (indirect calorimetry result/estimation by Cunningham formula), muscle strength, VO_2 max, biochemical variables; haemoglobin, glycated haemoglobin, ferritin, Calcium (Ca++), Magnesium (Mg++), Phosphate, Urea, Albumin, lipids, and sex hormones.

Measurement procedures

The vitamin K content of Jarlsberg cheese was determined by TINE SA using a method described previously.[13] Vitamin K in blood samples was analyzed as described previously.[13] As in previous studies the Osteocalcin analyses were performed at Vitas AS laboratories in Oslo. ucOCand uCOC were measured in plasma by immunoassay kits from Takara Bio Ōtsu.[13-15]

BMD: Participants were weighed in their underwear, and the height was measured with a fixed stadiometer (Seca scale, Mod: 8777021094, S/N: 5877248124885, Hamburg, Germany). A three-site dual energy X-ray absorptiometry scan (Lunar iDXA, GE Healthcare, Madison, WI) of lumbar area (L1-L4); femoral neck, trochanter, and proximal femur; whole body was performed to measure BMD and body composition following the Best Practice Procedure.[20] RMR was measured by indirect calorimetry.[21] Muscle strength: Maximal upper body muscle strength was evaluated by seated pull-down using a Technogym Radiant apparatus and lower body muscle strength by half-squat performed in a Smith machine as described for XCS.[22] VO_2 max was measured by an incremental VO_2max test running on a treadmill to volitional exhaustion at a 6^O incline and initiated at a speed of 7 km/h in the female, and 8 km/h in the male group. The test progressed with increases of 1 km/h every minute until the participant gave hand signal of 0.5 km/h increase or no further increase. The highest continuous VO_2 during a 60 second period was defined as VO_2 max.[23]

Statistical analysis

The continuously distributed variables were expressed by mean values with 95% confidence interval (CI). Standard deviations (SD) were given as an index of dispersion. Categorized variables were given in contingency tables.[24] Changes within and differences between groups were performed two-tailed and classified as significant for $p \leq 0.05$. Changes within groups on continuously distributed variable were performed by using paired t test and between group by analysis of variance (ANOVA).[25] The increase in tOC from baseline is assumed to be ordinal and the probability increases monotonically in a limited cheese dose interval. Isotonic regression was used for estimation of OED in the RSP-model.[26] Statistical Analysis System (SAS version 9.4) was used in the data analysis.

RESULTS

The mean daily Jarlsberg cheese dose in the first design level was 44.2g (range: 41.4-46.9) in females and 46.4 (range: 45.9-47.0) in males. During the second and third design level, the mean daily intake of Jarlsberg cheese in

females increased to 57.1 g (range: 51.1-62.5) and 65.4 g (range: 60.0-68.9), respectively. In male XCS the mean daily intake of Jarlsberg cheese increased to 56.2 (range: 37.5-62.5) and 70.2 g (range: 58.4-78.1) in the second- and third design level, respectively. The mean tOC in the total sample increased significantly ($p \leq 0.05$) from 35.2 (95% CI: 27-34.4) at baseline to 39.3 (95% CI: 30.1-48.5) at the end of the third design level (Figure 1).

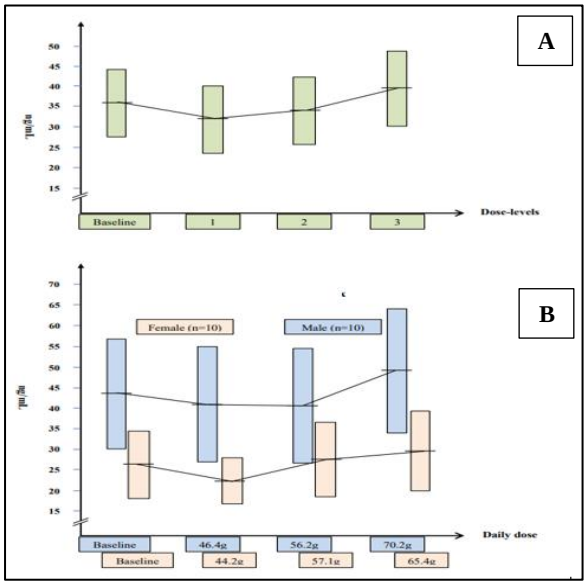

Figure 1: Development in Total Osteocalcin. The boxes show 95% confidence intervals of the mean indicated by the horizontal lines crossing the boxes, A) The development in the total sample (N=20) and B) divided in gender groups of (n=10).

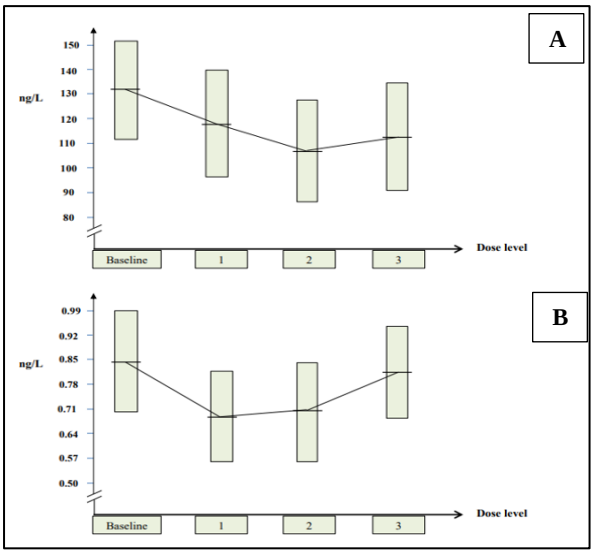

Figure 2: Development in Bone turnover Markers in the total sample (n=20); The boxes show 95% confidence intervals of the mean indicated by the horizontal lines crossing the boxes. (A) The development in PINP-1 and (B) the development in CTX-1.

In the female group, the tOC level was reduced from 26.5ng/ml (95% CI: 18.5-34.4) to 22.6 ng/ml (95% CI: 16.8-28.5) during the first design level, but increased to 27.3ng/ml (95% CI: 18.8-35.9) during the second design level and ending on 29.5ng/ml (95% CI: 20.2-38.7) during the third design level (Figure 1). The daily OED of Jarlsberg cheese for female XCSs was estimated to be 73.3 g (95% CI: 70.4-76.2), predicting a tOC level of 32ng/ml (95% CI: 22-42). The development in TOC in males was nearly parallel to the development in females (Figure 1).

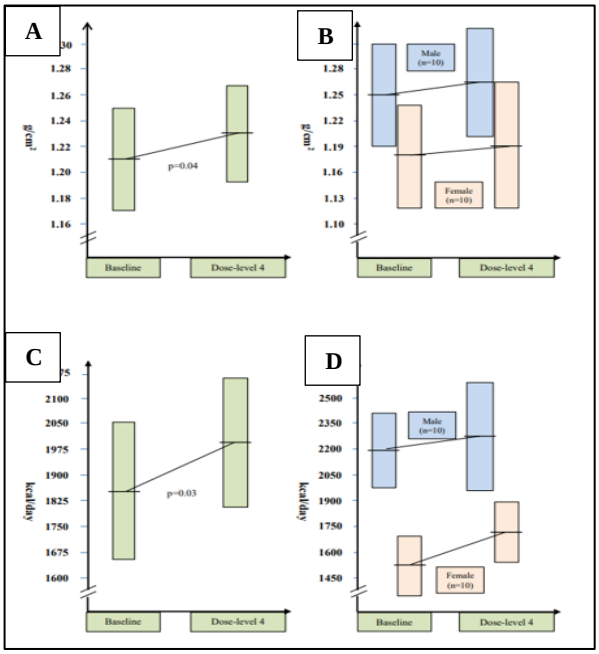

Figure 3: Development in bone mineral density and resting metabolic rate. The boxes show 95% confidence intervals of the mean indicated by the horizontal lines crossing the boxes, A) BMD development in the total sample (n=20), B) divided in gender groups of n=10, C) shows RMR development in the total sample and D) divided in gender groups of n=10.

The baseline level of 44.0ng/ml (95% CI: 31.2-56.8) was reduced to 41.7 ng/ml (95% CI: 28.0-55.5) during the first design level. No change was observed during the second level but increased to 49.2 ng/ml (95% CI: 34.3-64.0) during the third design level. The daily OED of Jarlsberg cheese for male XCSs was estimated to be 84.2 g (95% CI: 80.3-88.1), predicting a tOC level of 54 ng/ml (95% CI: 31-78).

The J-sharp development in tOC characterized by an initial decrease followed by a monotonic increase was detected in nine of 10 female and nine of 10 male XCS. The development in cOC followed the same pattern as described for tOC (Table 1). The cOC was reduced in the total sample from baseline during the first design level but increased during the second and third design level.

Table 1: Development in osteocalcin, vitamin K2, collagen, procollagen and bone-specific ALP within sex.

Parameters	Sex	Baseline	Week 4 (Level 1)	Week 8 (Level 2)	Week 12 (Level 3)	Week 12 Baseline
Carboxylated osteocalcin: cOC (ng/ml)	Female (N=10)	107.4 36.4) 81.4-133.5	90.8 (23.6) 74.0-107.7	99.1 (31.4) 76.6-121.6	110.1 (37.4) 83.4-136.8	2.7 (19.6) -11.4-16.7
	Male (N=10)	157.1 (65.9) 110.0 -204.3	138.7 (59.7) 96.0-181-3	138.9 (58.5) 97.0-180.8	157.7 (58.6) 115.7-199.6	0.5 (24.2) -16.7-17.8
Under- carboxylated osteocalcin ucOC (ng/ml)	Female (N=10)	8.4 (4.7) 5.1-11.8	7.3 (3.0) 5.1-9.4	10.5 (6.0) 6.3-14.8	10.7 (5.2) 6.9-14.4	2.2 (3.0) -0.1-4.4
	Male (N=10)	15.7 (10.7) 8.0-23.4	16.7 (11.5) 8.4-24.9	15.1 (10.5) 7.6-22.6	19.7 (12.9) 10.5-28.9	4.0 (8.3) -2.0-10.0
R_O= cOC/ucOC Osteocalcin	Female (N=10)	14.6 (4.1) 11.7-17.5	13.6 (3.4) 11.1-16.0	10.9 (3.6) 8.3-13.4	11.4 (3.0) 9.3-13.6	-3.2 (2.8) -5.2- -1.2
	Male (N=10)	12.8 (5.5) 8.9-16.8	10.5 (3.8) 7.8-13.3	12.4 (5.8) 8.2-16.5	9.3 (2.8) 7.4-11.3	-3.5 (4.8) -6.9- -0.1
Vitamin K2: MK-7 (nmol/l)	Female (N=10)	0.08 (0.05) 0.05-0.12	0.09 (0.03) 0.07-0.11	0.11 (0.06) 0.07-0.16	0.09 (0.03) 0.07-0.12	0.01 (0.03) -0.01-0.03
	Male (N=10)	0.11 (0.06) 0.07-0.16	0.14 (0.07) 0.09-0.18	0.14 (0.09) 0.07-0.20	0.08 (0.04) 0.05-0.11	-0.04 (0.06) -0.08-0.01
Vitamin K2: MK-8 (nmol/l)	Female (N=10)	0.05 (0.07) 0.00-0.11	0.06 (0.05) 0.02-0.09	0.13 (0.07) 0.07-0.18	0.10 (0.06) 0.06-0.15	0.05 (0.09) -0.02-0.11
	Male (N=10)	0.05 (0.03) 0.02-0.07	0.10 (0.10) 0.03-0.17	0.16 (0.12) 0.08-0.25	0.08 (0.07) 0.03-0.13	0.04 (0.07) -0.1-0.09
Vitamin K2: MK-9 (nmol/l)	Female (N=10)	0.16 (0.08) 0.10-0.22	0.16 (0.05) 0.12-0.20	0.15 (0.10) 0.08-0.23	0.12 (0.06) 0.07-0.16	-0.05 (0.06) -0.09-0.00
	Male (N=10)	0.15 (0.06) 0.11-0.19	0.21 (0.11) 0.13-0.29	0.18 (0.10) 0.11-0.25	0.08 (0.05) 0.04-0.12	-0.07 (0.08) -0.13- -0.01
Vitamin K2: MK-9 (4H) (nmol/l)	Female (N=10)	1.45 (0.25) 1.27-1.63	0.98 (0.34) 0.74-1.23	0.70 (0.27) 0.50-0.89	0.42 (0.06) 0.38-0.46	-1.03 (0.23) -1.19- -0.87
	Male (N=10)	0.95 (0.25) 0.78-1.13	0.77 (0.22) 0.61-0.93	0.63 (0.09) 0.57-0.70	0.32 (0.12) 0.23-0.41	-0.63 (0.33) -0.87- -0.40
PINP Procollagen (ng/ml)	Female (N=10)	107 (23) 90-124	91 (19) 78-105	83 (22) 67-99	92 (25) 74-110	-15 (12) -24- -6
	Male (N=10)	158 (55) 118-197	146 (50) 110-182	132 (48) 98-166	133 (52) 96-171	-24 (29) -45- -4
CTX Collagen (ng/ml)	Female (N=10)	0.66 (0.17) 0.54 – 0.78	0.55 (0.18) 0.42-0.67	0.55 (0.16) 0.43-0.66	0.71 (0.18) 0.58-0.84	0.05 (0.14) -0.05-0.15
	Male (N=10)	1.02 (0.33) 0.78-1.25	0.81 (0.27) 0.62-1.01	0.85 (0.34) 0.61-1.10	0.93 (0.64) 0.64-1.21	-0.09 (0.27) -0.28- 0.10
Bone-Specific ALP (ng/ml)	Female (N=10)	15.8 (5.2) 12.1-19.5	18.4 (5.5) 14.5-22.3	16.5 (4.6) 13.2-19.7	14.7 (4.7) 11.3-18.0	-1.1 (2.4) -2.8- 0.6
	Male (N=10)	27.1 (16.4) 15.4-38.9	31.3 (15.6) 20.1-42.5	29.7 (16.0) 18.3-41.1	23.2 (11.8) 14.7-31.7	-3.9 (5.3) -7.7- -0.1

The results are expressed by mean values with Standard Deviation in bracket and 95% Confidence Interval.

The development in cOC was similar in the two sex groups. The mean ucOC level at baseline decreased during the first design levels but increased significantly during the second and third design level (p=0.035), and similar pattern was found in both groups. The ucOC level was lowest among females (Table 1). The Osteocalcin ratio R_O was monotonically and significantly (p<0.01) reduced with 0.65 (Table 1). The sum of vitamin K_2 was reduced in the total sample from 1.51 ng/l (1.30-1.71) at baseline to 0.66 ng/l (0.54-0.77) at the end of the study (p<0.01). The development in total vitamin K_2 was equal in the two sex groups. No significant changes during the study were detected in MK-7 (Table 1). MK-8 increased (p=0.03) whereas vitamin MK-9 and MK-9 (4H) decreased significantly (p<0.01) in the total sample during the study with similar development within both sex groups. The mean PINP level in the total sample was significantly (p<0.01) reduced from 132ng/ml (95% CI: 112-152) at baseline to 118 ng/ml (95% CI: 97-140) and 108 ng/ml (95% CI:87-128) during the first and the second design level, respectively, but increased during the third design level to 113 ng/ml (95% CI: 91-134). (Figure 2). The development in PINP in the two groups showed similar patterns (Table 1). Mean CTX-1 in the total sample at baseline was 0.84 ng/ml (95% CI: 0.69-0.99) and ended on 0.82 ng/ml (95% CI: 0.67-0.97) after the third design level. CTX-1 was significantly reduced during the first but increased (p=0.04) during the third design level (Figure 2).

Table 2: Change in bone mineral density, resting metabolic rate ratio, muscle strength and VO2 from baseline to the end of the last design level (week 12).

Item	Sex	Baseline	Week 12	Increase
BMD, g/cm^2 L1-L4	Both, N=20	1.19 (0.10)	1.20 (0.10)	0.01 (0.02)
		1.14-1.23	1.15-1.24	0.00-0.02
	Female, N=10	1.18 (0.09)	1.18 (0.09)	0.01 (0.02)
		1.11-1.24	1.12-1.24	-0.01-0.02
	Male, N=10	1.19 (0.11)	1.21 (0.11)	0.02 (0.01)
		1.11-1.27	1.13-1.29	0.01-0.03
BMD, g/cm^2 Femur neck left	Both, N=20	1.11 (0.13)	1.11 (0.13)	0.00 (0.03)
		1.05-1.18	1.05-1.17	-0.02-0.01
	Female, N=10	1.11 (0.17)	1.11 (0.13)	0.00 (0.03)
		0.99-1.23	1.01-1.21	-0.02-0.01
	Male, N=10	1.12 (0.08)	1.12 (0.09)	0.00 (0.03)
		1.05-1.18	1.04-1.19	-0.02-0.02
BMD, g/cm^2 Femur neck right	Both, N=20	1.10 (0.12)	1.10 (0.13)	0.00 (0.04)
		1.04-1.16	1.04-1.16	-0.02-0.02
	Female, N=10	1,10 (0.16)	1.10 (0.16)	0.00 (0.02)
		0.99-1.22	0.99-1.21	-0.02-0.01
	Male, N=10	1.10 (0.08)	1.09 (0.10)	0.01 (0.06)
		1.03-1.16	1.02-1.16	-0.04-0.05
RMR ratio	Both, N=20	1.06 (0.18)	1.13 (0.16)	0.07 (0.16)
		0.97-1.15	1.06-1.20	0.00-0.15
	Female, N=10	0.99 (0.17)	1.11 (0.17)	0.12 (0.14)
		0.87-1.11	0.99-1.23	0.02-0.22
	Male, N=10	1.13 (0.17)	1.15 (0.16)	0.02 (0.18)
		1.01-1.25	1.04-1.26	-0.11-0.15
Muscle strength (kg), upper body	Both, N=19	34.9 (9.2)	37.2 (8.6)	2.9 (2.0)
		30.6-39.2	33.1-41.4	1.9-3.9
	Female, N=10	27.0 (3.9)	30.0 (3.3)	3.0 (2.0)
		24.2-29.8	27.6-32.4	1.6-4.4
	Male, N=9	42.8 (5.2)	45.2 (4.1)	2.7 (2.2)
		39.0-46.5	42.1-48.4	1.0-4.4
Muscle strength (kg), lower body	Both, N=20	141.9 (35.0)	150.3 (37.6)	11.8(10.0)
		125.5.158.3	131.6-169.0	6.8-16.9
	Female, N=10	115.3 (19.2)	126.6 (17.8)	11.3 (10.9)
		101.6-129.0	113.9-139.3	3.5-19.1
	Male, N=10	168.5 (25.4)	180.0 (34.9)	12.5 (9.6)
		150.3-187.7	150.9-209.1	4.4- 20.6
Max VO2, ml/kg BW/min	Both, N=18	67.4 (7.0)	70.9 (7.7)	2.8 (4.5)
		64.2-70.7	67.1-74.7	0.5-5.0
	Female, N=9	63.3 (6.3)	66.4 (5.4)	2.3 (2.5)
		58.7-67.8	62.3-70.6	0.4-4.2
	Male, N=9	71.6 (4.9)	75.4 (7.1)	3.2 (6.0)
		68.1-75.2	69.9-80.7	-1.4-7.8

The results are expressed by mean values with Standard Deviation in bracket and 95% Confidence Interval.

CTX-1 increased slightly among the females and decreased slightly among the males during the study (Table 1). These changes were not significant. The mean BAP in the total sample increased from 21.5 ng/ml (95% CI: 15.3-27.6) at baseline to 24,8 ng/ml (95% CI:18.7-31.0) during the first design level, and then decreased (p=0.02) during the two next levels, ending on 18.9 ng/ml (95% CI: 14.3-23.5) after the third design level (Table 1). Total BMD increased significantly (p=0.04) from baseline, representing an increase of 0.9% (0.1-1.6), (Figure 3) in both sex groups (Figure 3). Similar results were also detected for the lumbar (L1-L4) BMD which increased significantly (p=0.03) by 1% (95% CI:0.3-1.7). No significant changes in BMD were detected in the femur necks (Table 2).

The RMR and RMR-ratio increased (p=0.02; p=0.05) from baseline to the end of the study in the total sample (Figure 3). Both variables developed equally in the two sex groups (p= 0.02; p=0.06) (Figure 3). The muscle

strength in both the upper- and the lower body and VO_2-max increased significantly ($p<0.01$; $p<0.01$; $p=0.02$) in the total sample during the study. These variables developed equally in the two gender groups (Table 2). Ca^{++}, Mg^{++} and S-Albumin were significantly ($p=0.04$; $p<0.01$; $p=0.01$) reduced from baseline to the end of the study whereas, s-Urea, s-Phosphate, and vitamin D increased significantly ($p=0.02$; $p=0.03$; $p<0.01$) (Table 3). In the female group, the LH, Oestradiol and Progesterone increased during the study while FSH remained unchanged. Total testosterone increased in the male group. No significant changes were detected in body weight or BMI from baseline to the end of the study. No adverse events (AE) related to the investigational product were reported during the study.

Table 3: Biochemical- and hormone variables, the results are expressed by mean values (SD) and 95% confidence interval.

Variables	Baseline	Week 12	P value
S-Calcium (mmol/l)	2.37 (0.39) 2.33-2.42	2.34 (0.07) 2.31-2.38	0.04
S-Magnesium (mmol/l)	0.83 (0.05) 0.81-0.85	0.80 (0.05) 0.78-0.82	<0.01
S-Phosphate (mmol/l)	1.19 (0.12) 1.13-1.24	1.25 (0.13) 1.20-1.31	0.03
S-Urea (mmol/l)	6.0 (1.3) 5.1-6.9	6.9 (1.6) 6.1-7.6	0.02
S-Albumin (g/l)	44.7 (1.4) 43.7-45.7	44.1 (1.8) 43.2-44.9	0.01
LH (IU/l)	6.3 (2.6) 4.5-8.1	9.3 (11.4) 1.1-17.5	0.11
FSH (IU/l)	6.1 (2.4) 4.4-7.8	6.1 (1.4) 5.1-7.1	1.0
Estradiol (nmol/l)	0.21 (0.17) 0.09-0.33	0.31 (0.30) 0.10-0.53	0.18
Progesterone (nmol/l)	3.60 (1.90) 2.24-4.96	3.00 (0.00) -	0.21
Testosterone (nmol/l)	16.4 (3.3) 14.1-18.7	18.7 (4.7) 15.3-22.1	0.12

DISCUSSION

The primary aim of this study was to estimate OED of Jarlsberg Cheese to increase the tOC and the cOC in female and male XCS. Compared to the healthy fertile women in our previous cheese studies, the estimated OEDs were 24% and 30% higher for female and male XCS, respectively.[13] In the previous studies the participants were recreationally active women (Tier 1), and an average age of 37.5 years, and therefore lower degree of bone turnover and with lower energy and nutritional demands.[17,27,28] This may indicate that OED of Jarlsberg cheese is increased by high level of physical activity and reduced by age. The values of tOC and cOC at baseline were higher in the XCS compared to premenopausal women, even after a resting period with presumptively less bone turnover than during the competitive season. It is well known that the tOC levels of adolescents are higher than in adults, and higher in persons with high bone turnover.[27,28] The high levels of OC, PINP and CTX-1 at baseline reflect a high bone turnover, due to continuously bone repair and remodelling in the athletes. In the present study the development of TOC also showed a different pattern compared to the previous studies.[13-15] The OC levels decreased from high levels during the first two design levels, and then increased to a level above baseline at the third design level, forming a J-shaped curve. A previous dose-response study on premenopausal women showed an increase in OC development up to the OED and then flattened out by further increase of the Jarlsberg cheese dose.[13]

In the previous studies Jarlsberg cheese consumption caused significant increase in serum vitamin K_2.[13-15] In the present study only MK8 showed an increase. The significant increase in serum MK-8 demonstrates the absorption of Jarlsberg cheese derived vitamin K2. However, MK-9 (4H) was extraordinarily high among the XCS and, unlike previous clinical trials, decreased throughout the study. The opposite trends of MK-8 and MK-9 (4H) indicate additional sources of sMK-9(4H) than the cheese in this study, e.g., the gut microflora Marathon runners have been found to have a microbiota enriched in bacteria converting lactate to propionate, in particular post marathon.[29] Lactate to propionate conversion is the hallmark of *Propionibacterium species*, as is MK-9 (4H) production. The high MK-9 (4H) levels of the XCS may reflect diet independent absorption from a gut bacterium whose abundance is altered in response to heavy exercise. The reason for decrease in MK-9(4H) could possibly be enhanced conversion of long chained MKs into the bioactive MK-4 eating Jarlsberg cheese. The secondary variables PINP and CTX-1 followed a J-shaped pattern like the TOC-development. In a previous study, the PINP increased, leaving CTX-1 unchanged.[15] In this study BAP increased in the two first levels and then decreased below baseline. PINP is the best marker of osteoblastic activity, much more dynamic than BAP.[30] With few exceptions PINP and CTX-1 are elevated in cases of high bone turnover and reduced in cases of low bone turnover.[30] The XCS in the present study have higher baseline levels of PINP and CTX-1 than premenopausal women due to a higher bone-turnover.[31]

The effects of different antiresorptive regimens (ART) used in treatment of osteoporosis are reflected by a decrease of both PINP and CTX-1, and there will also be a decrease of BAP.[30,32] Anabolic treatment like PTH analogues stimulates bone anabolism increasing the BMD, reflected by increase of the PINP.[32] PINP and CTX-1 are the recommended BTMs to supervise the effect of antiresorptive treatment because they respond much faster due to effect than DXA.[30] The changes of BTMs in the present study were remarkable. Effects of resistance training and jumping intervention have been shown to improve BTMs and BMD in both elderly and young persons.[33-36] Distance running may be beneficial

for bone strength in adolescent men but not in adolescent women.[36] In this study no training intervention was performed other than allowing each participant to continue their normal training procedure. The elevation of BAP in the first two levels demonstrates activation of osteoblasts, while the decrease in PINP and CTX-1 is like the effect of ART, but at the end of the study shows a development like the effect anabolic treatment by PTH-analogues.[30,32] Another explanation to this might be higher training load and bone turnover during the last design level. The athletes were provided vitamin D to ensure calcium absorption, but no calcium supply. Even though the cheese is rich in calcium and magnesium, the levels decreased significantly. These results suggest that there is a suppression of osteoclasts and a stimulation of the osteoblasts. Furthermore, the significant decrease in both the Mg++ - and Ca++- levels and an increase of BAP also indicate stimulation of the osteoblasts. In a previous study comparing the effect of Jarlsberg to Camembert, a cheese not containing Vitamin K_2, we found an increase in Mg++ and Ca++ levels in the Camembert-group even if Jarlsberg cheese contains more Ca++ and Mg++ than camembert.[15] The levels in the Jarlsberg group decreased.[15] Osteoclastic differentiation and activation are regulated by an intricate cooperation in cytokine networks, involving osteoclastogenic and anti-osteoclastic cytokines.[37,38] Vitamin K_2 and DHNA have been shown to supress osteoclast genesis in vitro.[12,37,38] MK 7 is described to have higher bioavailability and longer half-life than other K vitamers.[39] MK 7 has been reported to increase bone quality and strength in animal studies, but the effect on BMD in humans are not sufficiently supported by clinical trials. Collagen production, and thus, bone quality and strength may be affected by MK-7 or MK-4 converted from MK-7.[39] MK-9 (4H) is the main K_2 vitamer in Jarlsberg cheese. The half-life and bioavailability of this vitamer has not been studied. The results in our previous cheese studies indicate that MK-9 (4H) has sustained bioactivity.[11,13,15,38] *Propionebacterium freudenreichii* is shown to be anti-inflammatory reducing the TNFα, and thereby possibly its activating effect on the osteoclasts.[40] In the present study the second DXA-scan was performed after only 12 weeks. The duration of a bone remodelling cycle is approximately 4 months, and there is a degree of lag before new bone can be detected radiologically.[41] However, both the total BMD and the lumbar BMD increased significantly. This result suggests a positive effect of Jarlsberg cheese on BMD underlined by the PINP, CTX-1, OC and BAP development combined with the decreased levels of Ca^{++} and Mg^{++}. The fast changes in electrolytes and bone markers while consuming Jarlsberg Cheese suggest both anabolic and anticatabolic effect on bone tissue in this study sample. As in the earlier studies, an increase of phosphate and urea was detected.[13-15] This is due to high protein level of the cheese and high protein turnover in muscle tissue. Although the athletes were very compliant to their "prescribed" cheese intake, filling in their cheese compliance form every day, they in general underreported their calorie intake in their dietary application. They reported such a low intake that it is not compatible with stable weight and energy consumption during exercise. However, changes in BTMs, RMR, muscle strength and VO2 max in the present study indicate that the athletes were in an anabolic phase not solely due to their dietary intake. The RMR increased in both sexes during the study. This might be affected by several factors. The study started in May, after a resting period after their competition season, but still attending school and having exams. During the two last levels they had summer holidays, and time to rest. The amount of physical exercise increased during the study period. We cannot conclude that the intake of Jarlsberg cheese is the cause of the positive development of RMR, but rather believe it reflects a positive development of energy balance.

Muscular strength has been shown to be improved by increased OC in elderly women, and OC seems to be an important factor preparing the body for a physical task.[8] The muscular strength and VO2 max increased significantly. This is most likely to be an effect of increased training load after a resting period from the end of March to May. Further controlled clinical studies should be performed with OED of Jarlsberg cheese to state the effect of cheese-induced osteocalcin increase on muscular strength, RMR, VO_2 max and the effect on BMD. A time limitation is that the study must be planned from the end of the competition season until the next, from April till November. More resources must be used to ensure compliance in diet registration, which in this study in general was poor although cheese compliance was good. Additionally, a long-term study will reveal if OED of Jarlsberg cheese may be recommended as a valuable supplement to exercise to prevent LEA-related bone loss. Although positive effects on blood lipids were detected in the previous studies, no significant changes were found in this study.[13-15] The slight increase in HDL may be due to high volume of endurance training.

CONCLUSION

In conclusion, the daily OED of Jarlsberg Cheese to increase TOC was estimated to be 73.3 grams per day for female XCS, and 84.2 grams per day for male XCS. The changes in OC, BTMs and BMD after cheese consumption suggest an antiresorptive and perhaps an anabolic effect of Jarlsberg Cheese on bone tissue. The VO2 max, RMR and muscular strength development indicated an anabolic situation more likely caused by other factors than cheese intake.

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