RESEARCH PROTOCOL

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Title

Efficiency Comparison of Jarlsberg- and Norvegia cheese on Osteocalcin and other Bone Markers; Estimation of the Jarlsberg cheese Effect on Resting Metabolic Rate, Bone Mineral Density, Muscle Strength and Peak VO2 in Cross-Country Skiers of both Sexes

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- Norwegian Research Council

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List of Abbreviations

AE	Adverse Event
ANOVA	Analysis of Variance
BALP	Bone specific alkaline phosphatase
BMD	Bone mineral density
CRF	Case Report Form
cOC	Carboxylated Osteocalcin
CTCAE	Common Toxicity Criteria
CTX-1	Collagen
DEXA	Bone Densitometry by X Ray
DH	Drammen Hospital
DHNA	1,4- Dihydroxy naphthoic Acid
DM	Data Management
FSH	Follicle stimulating hormone
GCP	Good Clinical Practice
ID	Identification
IEC	Independent Ethical XCS Committee
LH	Luteinizing hormone
MS	Muscle Strength
NMBU	Norges Miljø- og Biovitenskaplige Universitet
OED	Optimal Efficacy Dose
OR	Osteocalcin ratio
PI	Principal Investigator
PINP	Procollagen
PTH	Parathyroid hormone
RSP	Response Surface Pathway design
ucOC	Undercarboxylated osteocalcin
SAE	Serious Adverse Event
SAS	Statistical Analyses System
SD	Standard Deviation
SMC	Skjetten Medical Centre
SOP	Standard Operating Procedure
VO2	Peak Oxygen uptake
XCS	Cross-country skiers

Distribution of Clinical trial Protocol

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I: Synopsis

1.1: Project title

Comparison of Jarlsberg- and Norvegia cheese efficiency on Osteocalcin (OC) and Bone Turnover Markers (BTM); Evaluation of the effect of Jarlsberg cheese on BTM, Resting Metabolic Rate (RMR), Bone Mineral Density (BMD), Muscle Strength (MS) and Peak VO2 in Cross-Country Skiers (XCS) of both sexes

1.2: Protocol numbers

Protocol number: XCS-Jarlsberg/IIA
Regional Ethical Committee number: 537024
EudraCT number: 2022-003264-24
ClinicalTrial.gov: NCT05447936

1.3: Objectives

The study objective consists of the following two aims:

1. Compare the effect of daily intake of Jarlsberg and Norvegia cheese in change of the OC level and Carboxylated Osteocalcin(cOC), Bone Mineral Density (BMD), Trabecular bone score (TBS) and other bone markers in active XCS of both genders.
2. Compare the effect daily intake of Jarlsberg and Norvegia cheese in change of the Resting metabolic rate (RMR), Muscle Strength (MS), LM and Peak VO2 in active XCS of both genders.

1.4: Population and sampling

The study population consists of active XCS of both genders past 17 years of age for females and 18 years for males.

1.5: Design and randomization

The study is performed as an open randomized stratified parallel group designed trial with two strata. Gender is used as a stratification factor. The two equal sized strata are chosen because of different OED of cheese for women and men, and because the dose-response study previously performed has shown different response in men and women.

1.6: Study procedure

1.6.1 Screening:

The recruited XCS fulfilled the inclusion and avoiding exclusion criteria for the study, will undergo a screening investigation of 1 week. The candidate for participation fulfilling the criteria and signed the informed consent form for participation will be included in the study. Physical activity and diet registration will be performed. The week before the first investigation, the participants will be asked to fill in their diet for four days in a data application created for this study. The diet registration will be performed and monitored.

1.6.2 First comparison part:

The duration of the first comparative part is eight weeks. The XCS will randomly be allocated (1:1) to either daily intake of Jarlsberg cheese or Norvegia cheese for 8 weeks. The daily intake of Jarlsberg® cheese will be the sex-related estimated optimal efficacy dose (OED) for XCS and the comparative dose of Norvegia. The two cheese doses are nearly equal regarding total

energy, protein, carbohydrate, fat, and calcium. The trial cheese can be consumed with other food at breakfast, lunch, or other meals during the day. The daily doses of both cheeses will be 5 slices of cheese (75g/day) for females and 6 slices (90g/day) for males

The first clinical investigation in the study will take place and is denoted as Day 0. The participants will receive a study identification number and be informed not to use other cheese than the one received in the study. During this initial clinical examination, all demographic data, social factors, history of disease and vital signs will be recorded. Blood samples will be drawn fastening for measurements of Osteocalcin and vitamin K2; Haematological and biochemical variables like HbA1C and Lipids, magnesium, calcium, phosphate, and urea; Collagen (CTX-1) and procollagen (PINP), parathyroid hormone (PTH), and bone specific alkaline phosphatase (BALP); Estradiol and testosterone; LH, FSH and SHBG. Additionally, a DXA with VAT and Bodyfat (BF) investigation will be performed. In addition to BMD and TBS, the body composition variables Lean body mass (LM) and Fat free mass (FFM), will be measured together with RMR, muscle strength and Peak VO2 before starting the clinical part.

After four weeks the participants will meet for the second visit. Blood samples for measurements Osteocalcin and the specific bone turnover markers (BTM) will be drawn. The participants will be given a new sample of cheese and calcium tablets for the next four weeks and new date for the next visit.

Diet registration for four days will be performed in the data application every 8 weeks in the study. The diet registration will be monitored by a professional nutritionist.

The last investigation in this first comparative part of will take place at week 8. The participants will undergo a common clinical examination like the one performed initially. Blood samples for measurements of Osteocalcin, vitamin K2, and BMT will be taken. Haematological and biochemical variables except for vitamin D, parathyroid hormone (PTH), Estradiol and testosterone; LH, FSH and SHBG will be measured.

1.6.3 Second comparative part:

The duration of the second comparative part is 16 weeks and starts at study-week 8. The participants will receive new cheese, and calcium tablets every 4 weeks and meet for new clinical examination every 8 weeks.

The first clinical examination the this second comparative part will be performed at study-week 16. A diet registration will be performed the last week before the visit. A common clinical examination will be performed and blood samples for measurements of Osteocalcin, vitamin K2, the specific BTMs, sex hormones and biochemical variables will be drawn. The participants will be given new sample of cheese and calcium tablets for four weeks and a date for the next visit.

This visit performed at week 24 is the last in clinical examination in the study and equal to the initial study visit. Blood samples will be taken for measurements of Osteocalcin, BTMs, vitamin K2 and biochemical and haematological variables. Sex hormones, muscle strength, Peak VO2, DXA investigation for BMD, TBS and LM, and RMR will be measured in the same way as at day 0.

The female participants will be interviewed about their menstruation status including menarche length, cycles day and use of hormonal contraception.

1.6.4 Dietary Monitoring:

The participants will fill in their dietary intake in an application at baseline and every 8th week of the study. During the last visit an Eating Disorder Examination Questionnaire- Short, (Prenjak K, et al BMC Psychiatry 2020;20:1–7) derived from EDE-Q © Fairburn and Beglin, 2008 will be a part of the interview.

The questions will be woven into the anamnestic interview and not as a questionnaire to ensure that the participants do not suffer from disordered eating.

1.7: Main variables

The main variables in this study will be the Osteocalcin development, BTMs, RMR and BMD. Supporting variables are muscle strength, LM and Peak VO2 and gender hormones. The vitamin K2 variants MK-7, 8, 9, 9(4H), HbA1C, vitamin D, $Ca^{++,}$ Mg^{++} and urea, will be secondary variables. As safety variables, haematological- and biochemical variables and adverse events (AE) will be recorded at each visit. The participants' activity will be continuously recorded.

1.8: Sample size

With a significant level of 5%, a power of 90% and a clinically relevant difference in total osteocalcin increase of one-time SD between the two groups, at least 24 XCSs in each group must be included. By correcting for dropouts during the first part of the study, 30 XCS will be included in each group. Totally 60 XCSs will be recruited for participation.

1.9: Study duration

The duration of the study will be 24 weeks for each participant.

Recruitment period of XCS	01.01-31.05.23
Inclusion of the first participant	17th April 2023
Inclusion of the last participant	28th April 2023
Last participant finalized comparative study part	23rd June 2023
Last patient finalized follow-up part	14th Oct 2023

1.10: Flow Chart

	Comparison part I			Comparison part II	
	Week 0	Week 4	Week 8	Week 16	Week 24
Jarlsberg cheese group	x		x	x	x
Norvegia cheese group	x		x	x	x
Inclusion / Exclusion criteria.	x				
Patient factors history of disease	x				
Concomitant treatment	x	x	x	x	x
Diet registration	x		x	x	x
Vital signs	x				x
- Blood pressure	x				x
- Pulse rate	x				x
- Body weight	x				x
Blood sampling					
Bone specific markers	x	x	x	x	x
Osteocalcin-Vitamin K	x	x	x	x	x
-HbA1C & Lipids	x				x
-Vitamin D	x				x
Sex Hormones	x		x	x	x
-Haematological	x				x
-Biochemical	x		x	x	x
DXA; BMD, TBS, RMR	x				x
LM, MS & Peak VO2	x				x
Physical training	x		x	x	x
Menstruation status					x
Adverse Events [CTCAE]	x	x	x	x	x
Cheese compliance		x	x	x	x
End-of-study					x

II: Introduction

2.1: Background

Athletes competing in endurance sports are frequently exposed to episodes of Low Energy Availability (LEA) in association with the demands of their sport. It is proposed in the Relative Energy Deficiency in Sports (REDs), and the Female Athletic Triade, that LEA compromises bone health in both male and female athletes[1]. LEA is characterized by the perturbation of several hormones involved in the regulation of bone (re) modelling. LEA also causes menstrual disturbances; the most severe one is functional hypothalamic amenorrhea (FHA). This condition is reversible, but is considered a challenge for athletes, because either less training or increased energy intake is required. The highest prevalence of menstrual disturbances is seen in leanness and endurance sports such as running[2]. Not until recently the existence of LEA associated with bone loss was acknowledged in men, and therefore there are scarce data in male WBEA. Studies have shown that high-impact -exercise training can be highly osteogenetic with a low energy cost. Low repetition high-impact interventions have been shown to increase bone mineral density (BMD), cortical thickness and estimated strength, and preliminary evidence suggests that some of these effects may occur despite LEA[3]. Prolonged moderate impact exercise may help mitigate effects of short-term LEA, but it is unclear whether this would be of benefit to long-term bone health[3]. Given that bone health in endurance athletes can be compromised due to LEA, investigation of methods which may protect bone health in the face of LEA is of clinical importance[4].

The RED-S is a great problem in endurance sports. WBEA like cross country skiers often suffers from this condition. In case of muscular loss and bone stress injuries they miss a lot of training, and both their performance and their physical and mental well-being will suffer from this[1,6]. Cross Country Skiers (XCS) must train for several years to develop their full potential, and few other endurance sports (except for instance bicycling/swimming) have a higher demand for training volume to achieve this[7]. The procedures that have been suggested to treat LEA-related conditions have not been verified by controlled clinical trials, and further studies to clarify and improve our knowledge must be performed.

It is well established that dietary calcium and vitamin D are beneficial for skeletal health. Additionally, it has been documented that vitamin K has beneficial effects on the skeleton[7]. Activated or carboxylated osteocalcin has a key role in bone-formation [,8,9]. It is one of the 17 body's GLA proteins and activated in a process involving vitamin K_2. In Japan, vitamin K has been approved as an aid for treating osteoporosis, but the doses recommended are high and the efficiency is limited or absent[10].

Many dairy products are good calcium sources and cheese is an important source of vitamin $_{K2}$. This indicates that cheese consumption may strengthen bones, but the effect of eating cheese rich in vitamin K_2 have not been studied abundantly.

There are several vitamin K variants, and the long-chained vitamin K_2 variants have proven to be much more efficient in activating osteocalcin than short chained K_2 or K_1. In the western diet, fermented dairy products such as cheese are the best sources of long-chain vitamin K_2, and health benefits related to vitamin K in cheese have been reported[11,12,13,14]. Jarlsberg cheese has been shown to be particularly rich in vitamin K_2. In a recently published dose-response study,

aiming to estimate an optimal daily Jarlsberg dose related to carboxylation of osteocalcin, gave some unexpected and interesting results[15,16]. The carboxylated osteocalcin(cOC) and the osteocalcin ratio (R_0 = cOC/ucOC) increased significantly with increasing Jarlsberg dose up to 60 g/day before it flattened out and even declined. The surprise was that the total osteocalcin (tOC=cOC + ucOC) increased significantly with 46.1% after a few weeks of daily Jarlsberg cheese intake. These results were verified in our next study aiming to estimate a maintenance daily dose of Jarlsberg. To the best of our knowledge, no clinical study has previously showed that the OCN level can be increased by food. However, laboratory animal study has shown that 1,4-dihydroxy-2-naphthoic acid (DHNA) increases the tOC level and showed an anti-osteoporotic effect in ovariectomized mice with increasing of bone density and trabecular thickness[17].

In our comparative study the effect of Jarlsberg Cheese was compared with the effect of Camembert cheese not containing vitamin K2 and DHNA[18]. No differences were detected between the groups regarding haematological variables, but interesting differences were detected in the biochemical variables. Serum Calcium, Magnesium, and HbA1c were significantly reduced in the Jarlsberg group, but found unchanged in the comparative group. PINP**,** tOC, cOC, R_O and vitamin K_2 increased significantly ($p<0.01$) after 6 weeks in the Jarlsberg-group. PINP remained unchanged in the Camembert-group. The other variables decreased slightly in the Camembert-group but increased significantly ($p\leq0.05$) after switching to Jarlsberg®. No CTX-changes detected in neither of the groups. Serum triglycerides increased slightly in both groups. Switching to Jarlsberg, total cholesterol and LDL-cholesterol were significantly reduced ($p\leq0.05$). HbA1c, Ca^{++} and Mg^{++} were significantly reduced in the Jarlsberg-group, but unchanged in the C-group. Switching to Jarlsberg, HbA1c and Ca^{++} decreased significantly. The effect of daily Jarlsberg® intake on increased s-osteocalcin level is not a general cheese effect. Jarlsberg® containing vitamin K_2 and DHNA which increases PINP, tOC, cOC and R_O and decreases Ca^{++} and Mg^{++}. These effects reflect increased bone anabolism.

The dominating vitamin K_2 vitamer is MK-9 in most cheeses, but the amount varies considerably[19]. However, some cheeses also contain MK-9(4H). Jarlsberg cheese is rich in this compound[20]. This cheese is made with lactic acid bacteria producing MK-8 and MK-9 and *Propionibacterium freudenreichii* (PF) producing MK-9(4H). In the fermentation process to obtain the vitamin K_2 vitamer MK-9(4H), PF additionally produces DHNA. We have shown that Jarlsberg cheese contains both the vitamin K2 vitamers and DHNA. The results from our studies make Jarlsberg cheese consumption very promising and we argue that it should be investigated whether Jarlsberg Cheese might prevent reduced BMD.

Our previous studies have been performed on healthy premenopausal women between 25 and 50 years of age and the estimated daily optimal efficacy dose (OED) of Jarlsberg was 60 grams. The study population was chosen because most of the osteoporotic patients are women[16].

Osteoporosis represents substantial health related- and social challenges. Although bone loss is most common among women and elderly persons of both genders, it also affects the younger generation. Young athletes are frequently exposed to microfractures, and the syndrome of Relative Energy Deficiency in Sport (RED-S) with or without disordered eating, is as mentioned a widespread problem among athletes in weight-dependent sports such as

running and cross country skiing[3,21]. The syndrome causes impairment of metabolic rate, menstrual function, bone health, immunity, protein synthesis and cardiovascular health. The aetiological factor of this syndrome is low energy availability[2]. Our results, supported by other international studies[22-26], strongly indicate that the compounds of Jarlsberg cheese may have a promising prophylactic effect on reduced BMD and might affect lipid levels and glucose metabolism. Osteocalcin is showed to build strong bones by increasing the BMD, improve muscular strength, stimulate testosterone production; it has a beneficial effect on cardiac health, lipid pattern and adopositas[22-26]. Daily intake of Jarlsberg cheese may have a general positive impact on young athletes by protecting against different diseases due to the contents of DHNA and vitamin K_2. DHNA is showed to increase trabecular thickness in ovariectomized mice[17]. Cheese has not been regarded as a particularly healthy food because of the contents of milk fat. For young athletes, however the extra energy supply by the cheese is a positive factor in the face of LEA. We do not yet know if the Jarlsberg® Cheese could be used as a food supply to improve bone health, and we do not know if it could help young athletes to maintain or improve bone health.
Since young XCS are at risk for LEA and RED-S, we want to perform studies on XCS athletes.
Recently a dose response study on XCS was performed, and OED for female athletes was estimated to 70 grams of Jarlsberg ® Cheese, and 80 grams for male athletes to increase the tOC and cOC[27]. The effect of this OED should be tested. All previous studies show a significant decrease in serum magnesium and calcium, and a significant increase of serum urea and serum phosphate. The increase in BMD is not significant, but the increase in RMR is.

2.2: Osteocalcin and Vitamin K

Activated osteocalcin has a key role in bone formation and maintenance. It is one of the body's 17 so called GLA proteins, all of which being activated by carboxylation in a process involving vitamin K. While vitamin K dependent coagulation factors are practically fully carboxylated under normal conditions, osteocalcin is not. The ratio of fully carboxylated to under carboxylated osteocalcin in the blood (OR) reflects a person's vitamin K status. The high levels of under carboxylated osteocalcin in healthy people indicate that suboptimal vitamin K status or subclinical vitamin K deficiency is common in Western societies [23]. Very low ORs have been associated with osteoporotic fractures [26].
Figure 1 shows the structure of common forms of vitamin K. Vitamin K1 is produced in plants and found at high concentrations in leafy vegetables and is the dominating variant in the Western diet.

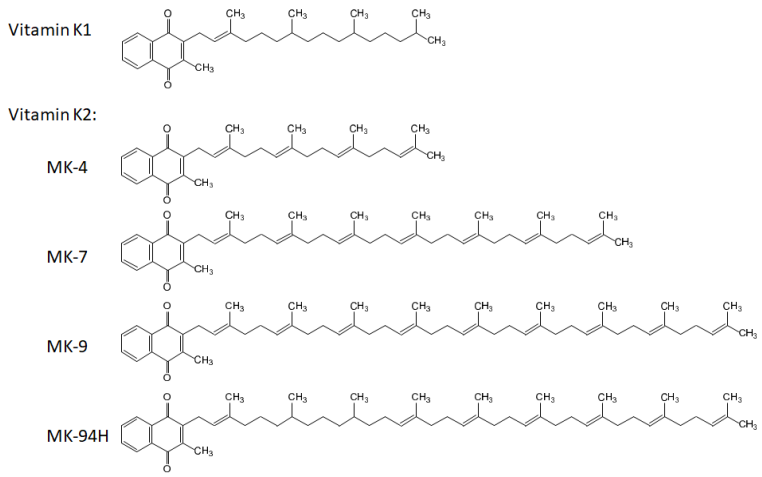


Figure 1: Structures of some vitamin K vitamers

Vitamin K_2 (menaquinone, MK) is found in several variants (Fig 1). The short-chained MK-4 can be formed from vitamin K_1 in humans and is found in animal products like liver. The K_2 vitamers with longer side chains, like MK-7, MK-8, MK-9, and MK-9(4H) are of bacterial origin. They can be found in certain fermented foods. In the Western diet fermented dairy products like cheese are the main source of vitamin K_2.
The long chained MKs have been found to have greater extra-hepatic activity than K_1 and MK-4, possibly due to more efficient uptake and much longer serum half-life [11,12].
Prospective cohort studies have demonstrated health benefits that can be attributed the intake of vitamin K_2 but not K_1, and the main contributor to the vitamin K_2 is cheese containing MKs with long side chains [13,14].

2.3: Jarlsberg versus Norvegia Cheese

The dominating K_2 vitamer is MK-9 in most cheeses, but the amount varies considerably[19].
However, some cheeses also contain MK-9(4H). Jarlsberg cheese is rich in this compound[20].
This cheese is made with lactic acid bacteria producing MK-8 and MK-9 and *Propionibacterium freudenreichii (PF)* producing MK-9(4H).
Although vitamin K_2 related health benefits have been associated with cheese, the effects of cheese consumption on bone health have never been studied in intervention studies. Because of its high vitamin K_2 content Jarlsberg cheese is well suited for such a study.
Recently, a dose-response study in healthy Norwegian women was performed with a 3-level between patient Response Surface Pathway design [15,16]. The study was performed in 19 women with daily intake of Jarlsberg cheese during a period of 5 weeks[15]. This study showed a significant increase of OC and cOC. Jarlsberg Cheese has been compared to Camembert cheese which does not contain any vitamin K_2, and no similar effects on the Osteocalcin level was detected after Camembert consumption[18]. Norvegia Cheese is very similar to Jarlsberg Cheese. The only differences are lack of MK-9(4H) and DHNA (Table I).

Table I: Vitamin K content of 100g a) Norvegia cheese and b)Jarlsberg cheese

a) Norvegia cheese

	MK-4	***K1***	***MK-7***	***MK-8***	***MK-9***	***MK-9(4H)***	***Σ vitamin K***
Mean concentration original Norvegia (n=10)	*4,1*	*3,7*	*1,6*	*8,9*	*25,7*	*n.d.*	**44,0**
relSTDEV between samples	*17 %*	*14 %*	*24 %*	*27 %*	*27 %*	*-*	*22 %*
Mean concentration low-fat Norvegia (n=10)	*2,5*	*2,6*	*1,1*	*6,1*	*18,7*	*n.d.*	***31,0***
Expanded uncertainty	*-15 / 17 %*	*-12 / 20%*	*-16 / 16 %*	*-12 / 49 %*	*-11 / 20 %*	*-11 / 21 %*	*-12/ 21 %*

b) Jarlsberg cheese

	MK-4	***K1***	***MK-7***	***MK-8***	***MK-9***	***MK-9(4H)***	***Σ vitamin K***
Mean concentration original Jarlsberg (n=21)	*5,0*	*2,9*	*1,6*	*9,0*	*25,6*	*40,8*	***85,0***
relSTDEV between samples original Jarlsberg (n=21)	*31 %*	*31 %*	*15 %*	*17 %*	*22 %*	*23 %*	*12 %*
Mean concentration low-fat Jarlsberg	*2,5*	*2,4*	*1,5*	*10,5*	*42,0*	*26,0*	***84,8***
Expanded analytical uncertainty	*-15 / 17 %*	*-12 / 20%*	*-16 / 16 %*	*-12 / 49 %*	*-11 / 20 %*	*-11 / 21 %*	*-12/ 21 %*

The amount of the vitamers MK-4,7,8 and 9 is equal in Jarlsberg and Norvegia Cheese but differs regarding MK-9(4 H). We want to study if this is the background for our previous results[15,16,18].

2.4: Objectives.

The study objective consists of the following two aims:

1. Compare the effect of daily intake of Jarlsberg and Norvegia cheese in change of the OC level and Carboxylated Osteocalcin(cOC) BMD and other bone markers in active XCS of both genders.
2. Compare the effect daily intake of Jarlsberg and Norvegia cheese in change of the Resting metabolic rate (RMR), Muscle Strength (MS) and Peak VO2 in active XCS of both genders.

III: Population and sampling

3.0: Study unit

The study unit is active athletes.

3.1: Reference population

Active athletes of both sexes from 17 years of age for females and 18 years for males representing sports with equal loading.

3.2: Study population

3.2.1: Inclusion criteria.

Active XCS from 17 years of age for females and 18 years for males.

3.2.2: Exclusion criteria.

Active XCS fulfils at least one of the following criteria will be excluded from participation in the study:

- Eating disorder
- Pregnancy
- Known gastrointestinal disorder.
- Abnormal liver or kidney function.
- Diabetes
- Suffering from verified cancer.
- Under systemic treatment with corticosteroids or other immunosuppressive drugs the last 3 weeks before start of the trial treatment.
- Participating in another clinical trial with pharmaceuticals the last six weeks before start of this trial treatment.
- Known milk product allergy.
- Suffering from diseases or injuries that disable them to perform VO2 or muscular strength tests.
- Not able to understand information.
- Do not want or not able to give written consent to participate in the study.

3.3: Recruitment of athletes

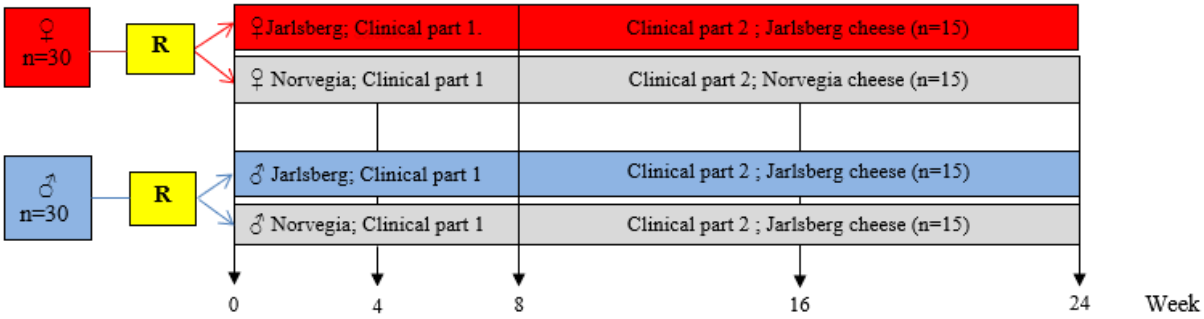
The active XCS will be recruited by the primary investigators team and by advertising in social media. They will be recruited from high schools of sports, and cross-country teams and clubs in the area around Oslo. Active XCS fulfils the inclusion and avoid the exclusion criteria will be asked to participate and to give written consent for participation.

IV: Design and randomization

4.1: Study design

The study is performed as an open randomized stratified parallel group design with two strata[28]. Sex is used as a stratification factor. The two equal sized strata are chosen because of different Optimal Effective Dose (OED) of cheese for women and men, and because the dose-response study previously performed has shown different response in men and women[29].

Within each stratum the participants will be allocated into two treatment groups by block randomisation.



4.2: Randomization

The athletes will be randomized (1:1) to either daily intake of Jarlsberg cheese or Norvegia cheese by using block randomization with random block size between 2 and 6[29]. The daily amount of cheese will be equal in the two cheese-groups with 5 slices (75g) for females and 6 slices (90g) for males.

14.3: Power Analysis

With a significant level of 5%, a power of 90% and a clinically relevant difference in total osteocalcin increase of one-time SD between the two groups, at least 24 XCSs in each group must be included. By correcting for dropouts during the first part of the study, 30 XCS will be included in each group. Totally 60 XCSs will be recruited for participation[29].

4.4: Identification of subjects

All the XCS will be given one study identification number of four digits constructed as follows:

Digit 1:	Indicate the gender study arm (1=female, 2=male)
Digit 2&3:	Indicate the athlete number consecutively within each sex arm (01,02,03 etc.)

V: Evaluation

5.1: Osteocalcin and vitamin K

The main response variable in both the first part of the two sex arms in the study will be tOC, the bone specific markers procollagen (PINP), Collagen (CTX1), Bone specific Alkaline Phosphatase (BALP) and parathyroid hormone (PTH). In the second part of the study, Bone mineral density (BMD) will be the most central variable in addition to Resting metabolic rate (RMR), Muscle strength and peak VO_2. The Osteocalcin ratio R_0, vitamin K_2 including the different vitamers MK 7, 8, 9 and 9/4H and vitamin K_1 will be used as secondary variables in both study parts.

5.2: Bone specific markers

The bone specific markers investigated in the study are Procollagen (PINP), Collagen (CTX1), Bone specific Alkaline Phosphatase (BALP), Parathyroid hormone (PTH).

5.3: Bone mineral density (BMD)

Participants will be weighed in their underwear, and height will be measured with a fixed stadiometer (Seca scale, Mod: 8777021094, S/N: 587728124885, Seca Deutschland, Hamburg, Germany). A dual energy X-ray absorptiometry (Lunar iDXA, E'CORE Software, version 14.10.022; GE Healthcare, Madison, WI) performing a three-site scan (lumbar area [L2-L4]; femoral neck, trochanter, and shaft [proximal femur]; and whole body compares the bone density with the bone density expected for a healthy adult of the same age, sex and ethnicity as the patient. Total body composition for different necessary measures will be performed. The difference is calculated as a standard deviation (SD) score. The difference between the patient measurement and that of a young healthy adult is known as a T score. The T scores classify as follows:

- above -1 SD is normal.
- between -1 and -2.5 SD is defined as mildly reduced bone mineral density (BMD) compared with peak bone mass (PBM)
- below -2.5 SD is defined as osteoporosis[30].

The difference between the participants measurement and that of someone of the same age and gender is known as a Z score. A Z-score below -2 indicate a bone density lower than it should be for someone of the participant's age. A Z-score is supposed to be 0, above 0 is very good, below -1 is poor. Z-Score < -1 is recommended further follow-up and clinical examination of secondary risk factors[31].

Trabecular Bone Score (TBS) will be calculated from the lumbar spine images using TBS iNsight software (Medimaps, Geneva, Switzerland) version 3.0.1. As there is no reference population for TBS in individuals < 40 years, only the absolute values will be used.

Evaluation of the microarchitecture of either tibia or femur neck would give even better information about the bone health of the athletes, and we are looking for possibilities[32].

Bodyfat (BF) investigation will be performed. In addition to BMD and TBS, the body composition variables Lean body mass (LM), Bodyfat (BF) and Fat free mass (FFM), will be measured together with RMR, muscle strength and Peak VO2 before starting the clinical part and at the end of the study.

5.4: Muscle strength

Maximal (1 repetition maximum, 1RM) upper body muscle strength will be evaluated by seated pull-down by use of a Technogym Radiant (Technogym, Gambettola, Italy) apparatus and by half-squat performed in a Smith machine (Technogym 2SC multipower, Gambettola, Italy). Test procedure will be initiated by three sets of exercise-specific warm-up with gradually increasing load (ten repetitions at 40%, six repetitions at 60%, and three repetitions at 80% of expected 1RM). The first attempt will be performed with a load approximately 5% below the expected 1RM. After each successful attempt, the load will be increased by 2–5% until the athlete fails to lift the load after 2–3 consecutive attempts. The rest period between each attempt will be 3–4 min. The movement for seated pull-down will start with the handlebar positioned at the same height as the forehead. The athletes must pull the handlebar down to the hip bone. Elbows must be held slightly lateral to simulate a double-poling pull, and the wire must be parallel to the back support on the bench. For the 1RM to be accepted, the handlebar must be pulled completely down in one continuous motion with hands parallel[33].

For half-squat, at the familiarization session, the correct depth (90° knee angle) will be noted for reproduction. The position of the feet will be marked, and the correct depth controlled with an elastic band[33,34].

For additional information about changes in muscle mass during the study, lean body mass (LM) will be extracted from the body composition outcomes obtained from the DXA measurement.

5.5: Peak VO_2

Peak VO2 will be measured by performing a cardiopulmonary exercise test on a treadmill (ELG 90/200 Sports; Woodway, Weil am Rhein, Germany) with an incremental protocol until exhaustion. Gas exchange will be measured using a breath-by-breath gas analysis system (Oxycon Pro analyzer; Jaeger, Würzburg, Germany) with a Hans Rudolph two-way breathing mask (2700 series; Hans Rudolph, Kansas City, KS). Each test is initiated by an individual adjusted warm-up of 15 minutes at 70% of HRmax. The incremental test protocol involves an incline of 10% with individual adjustment of load each minute until reaching exhaustion, resulting in a total duration of about 4-6 minutes with constant load the last minute. Measures of respiratory exchange ratio (RER) ≥ 1.10, and lactate concentration ≥ 7.0 mmol/L measured 1 min after test termination and analysed immediately in a 1500-Sport-lactate analyser (YSI, Yellow Springs Instruments, Yellow Springs, OH), were required to ensure a valid measure of maximal oxygen uptake (VO_2max). A Borg scale rating ≥ 17 may additionally be used to confirm the test result [35].

5.6. Resting metabolic rate (RMR)

RMR will be measured by IC using a respiratory gas analyser (Oxycon Pro, Jaeger, Germany). Ambient conditions are registered, and the analyser is gas and volume calibrated each morning prior to the measurements, according to the recommendations stated in the user manual from the manufacturer user manual for Oxygen Pro, Jaeger, Germany. Gas exchange and ventilator variables will be measured continuously using the breath-by-breath method. Participants will be instructed to rest for 10 min, wearing a two-way breathing mask covering their nose and mouth (2700 series: Hans Rudolph, Inc). Thereafter, the measurement period is started by connecting the mask to the gas analyser, and data collection continues for a total of

20 minutes. To get reliable results a steady state of 5 minutes of no more than 10% variation of gas exchange is required. The results from the RMR measurements will be related to the estimated RMR. The most reliable formula for estimating the RMR is the Cunningham formula; 22 x Fat free mass + 500. We will obtain the fat-free mass from the DXA scan. Ratio: measured/estimated RMR should be above 0,9.[36]

5.7: Laboratory variables

The haematological and clinical biochemical variables to be measured are listed below.

5.7.1 Biochemical variables:

Creatinine
Albumin
Urea
ALAT
Calcium
Magnesium
Phosphate
Ferritin
Vitamin D

5.7.2 Lipids:

Total Cholesterol
HDL cholesterol
LDL cholesterol
Triglycerides

5.7.3 Bone Turnover markers

Collagen 1 (CTX-1)
Procollagen 1 (PINP)
Parathyroid hormone (PTH)
Bone specific alkaline phosphatase (BALP)

5.7.4 Sex hormones:

Estradiol
Progesterone
Testosterone
LH
FSH

5.7.5: Haematology

Haemoglobin (Hgb)
HbA_1C

5.8: Diet registration

Diet registration will be performed by the participants. The daily intake of all food will be recorded daily for four days consecutively at screening and every eight weeks during the study. A pre-evaluated questionnaire based “diet.no” will be used and recorded on an electronical diet APP by the participants. The diet registration will be supervised by a qualified monitor. The results will be evaluated by the investigating team.

5.9: Menstruation status

To control the hormone blood sample results to female XCS, this group will be interviewed about their menstruation status. This includes menarche length, cycles day and use of hormonal contraceptives.

5.10: Cheese, supplement, and activity registration

The cheese, supplement of vitamin D and activity will med recorded in a form for daily registration. The activity will be recorded weekly. The parameters to be recorded are low- and high intensity running and roller-ski in hours, and low- and high-load strength exercise. The forms are handed out every fourth week of the study and collected by the investigator or the research assistant.

5.11: Compliance

The participants must eat their daily prescribed dose of cheese and vitamin D, register their activity weekly, and fill in their diet APP 4 days before their control every 8 weeks.

5.12: Adverse Events (AE)

5.12.1: Common Terminology Criteria for Adverse Events version 4.0 (CTCAE)

The CTCAE is divided in 26 System Organ Class (SOC) in accordance with the MedDRA hierarch. Within each SOC, AE are listed and accompanied by descriptions of severity or grade:

- Blood and lymphatic system disorders (11 Items)
- Cardiac disorders (36 Items)
- Congenital, familial, and genetic disorders (1 Items)
- Ear and labyrinth disorders (9 Items)
- Endocrine disorders (11 Items)
- Eye disorders (25 Items)
- Gastrointestinal disorders (117 Items)
- General disorders and administration site conditions (24 Items)
- Hepatobiliary disorder (16 Items)
- Immune system disorders (6 Items)
- Infections and infestations (76 Items)
- Injury, poisoning and procedural complications (78 Items)
- Investigations (38 Items)
- Metabolism and nutrition disorders (24 Items)
- Musculoskeletal and connective disorders (41 Items)
- Neoplasms benign, malignant and unspecified incl. cysts and polyps (5 Items)
- Nervous system disorders (63 Items)
- Pregnancy, puerperium and perinatal conditions (5 Items)

- Psychiatric disorders (20 Items)
- Renal and urinary disorders (20 Items)
- Reproductive system and breast disorders (51 Items)
- Respiratory, thoracic and mediastinal disorders (59 Items)
- Skin and subcutaneous tissue disorders (34 Items)
- Social circumstances (2 Items)
- Surgical and medical procedures (1 Item)
- Vascular disorders (17 Items)

5.12.2: Grading and classification

Grade refers to the severity of the AE. The CTCAE displays Grade 1 through 5 with unique clinical descriptions of severity for each AE based on this general guideline:

Grade 0 =	None
Grade 1 =	Mild: asymptomatic or mild symptoms; clinical or diagnostic. Observations only; intervention not indicated.
Grade 2 =	moderate: minimal, local, or non-invasive intervention indicated; Limiting age-appropriate instrumental Activity of Daily Living (ADL)
Grade 3 =	severe or medically significant but not immediately life-threatening: Hospitalization or prolongation of hospitalization indicated; disabling; Limiting self-care ADL
Grade 4 =	Life-threatening consequences; urgent intervention indicated
Grade 5 =	Death related to AE.

Relation to trial medication as: Definitely, Probably, Possibly or Unrelated
Action taken as: None, Interruption, Modified or Discontinued
AE treatment as: None, Continue Medication, Procedure or Hospitalization.
Outcome at last visit as: Resolved, Ongoing or Fatal.

Definitions of relationship to study treatment are as follows:

Unrelated:	bears no relation to timing of medication, like symptoms or signs expected in the disease process, does not recur on re-challenge.
Possibly:	bears relation to timing of medication, like symptoms or signs expected in the disease process, does not recur on re-challenge.
Probably:	bears clear relation to timing of medication, distinct from symptoms or signs expected in the disease process, does not recur on re-challenge.
Definitely:	bears clear relation to timing of medication, distinct from symptoms or signs expected in the disease process, recurs on re-challenge.

5.12.3: Reporting adverse events.

An adverse event (AE) is any untoward symptom or sign befalling a patient in a clinical trial regardless of its relationship to the study medications. All AEs must be described in detail and their severity and putative relationship to the study medication noted.
AEs may be considered serious. The definition of this is as follows:

- Death
- Life threatening
- Leads to or prolongs hospitalization

- Results in persistent of significant disability
- Any new important medical information.

5.13 Patient factors, Vital signs, and Physical examination.

The patient factors to be recorded in the study will be age in years from birth to the screening visit, height in cm and body weight in kg. Previous medical history, duration of any disease in months from the final diagnosis to the screening visit, previous and on-going treatment of the disease will be observed. During each physical examination, body weight, concomitant disease and treatment will be recorded. Additionally, vital signs defined as Systolic and Diastolic blood pressure in mmHg and Heart rate in beats/min will be recorded in sitting position after five-minute rest. To eliminate any vitamin D disturbances the participants are provided supplement of 40µg Vitamin D from the start till the end of the study.

VI: Study procedure

6.1: Definitions

Important definitions are:

1) Osteocalcin Ratio (R_0): cOC (carboxylated Osteocalcin)/ uOC (undercarboxylated osteocalcin).
2) Bone Mineral Density (BMD) and Trabecular Bone Score (TBS).
3) Lean Body Mass (LM) extracted from the body composition outcomes obtained from DXA.
4) RMR ratio (resting metabolic rate ratio). Observed RMR/ 22x FFM+ 500 (The Cunningham formula)
5) Peak VO2 which is maximal oxygen uptake.

6.2 Clinical procedure

6.2.1 Screening:

The recruited XCS fulfilling the inclusion and avoided the exclusion criteria will be registered and asked to participate in the study. The athletes who are willing to sign the informed consent form will be enrolled in the study. During this one week the participants will undergo a clinical screening and an appointment for the starting visit in the study one week later. Before the first investigation, the participants will be asked to fill in their diet for four days in a data application created for this study. The diet registration will be performed and monitored by a nutritionist. All the athletes will be instructed not to change their usual intake of food during the study but change the usual used cheese with the study cheese. During this initial clinical examination, all demographic data, social factors, history of disease and vital signs will be recorded.

6.2.2: Start-up Day:

A clinical investigation with blood sampling will be performed at the start-up denoted as Day 0, and they are all asked to meet fastening, and without hard training two days in front. Blood samples will be collected for measurements of Osteocalcin, vitamin K_2; Biochemical variables like; Ca, Mg, Na, potassium, urea, phosphate, creatinine, ferritin, haemoglobin, HbA1C, Lipids, CTX-1, PINP, BALP, PTH, Estradiol, Testosterone, LH, FSH and vitamin D. Additionally, DXA investigation of BMD and TBS, FFM and LM and RMR will be recorded, and muscle strength, and Peak VO_2 will be tested before starting the clinical part.
A time schedule for the inclusion visit day will be followed. The participants will meet to their appointment at NIH fasting from midnight. The two first examinations will be RMR and DXA measurements, and both lasts for 30 minutes. The two procedures are performed by qualified personnel at NIH. The next station fasting is blood sampling in the laboratory by a qualified bioengineer. After the blood sampling they will meet for medical interview by the primary investigator, recording ongoing and previous diseases, concomitant and former treatment, inclusion, and exclusion criteria, and signing the informed conscient form. The next step is examination of blood pressure, pulse rate and weight, and the medical session will be finished with handling and the cheese registration form, and their allocated sample of cheese type.
Blood samples will be handled in accordance with the laboratory centre procedures, and the Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 registered.
The next step in the program is then lunch and rest for approximately 2 hours.

After lunch they will start with the VO2 tests on treadmill and be sent for the muscular strength tests. Both VO2 test and muscular strength tests will be supervised by qualified master students.

6.2.3 Comparative phase 1:

The XCS will randomly be allocated (1:1) to either daily intake of Jarlsberg cheese or Norvegia cheese. The daily intake of Jarlsberg cheese will be the OED for XCS and the comparative dose of Norvegia. The two cheese doses are nearly equal regarding total energy, protein carbohydrate and fat magnesium and calcium. The trial cheese can be consumed with other food at breakfast, lunch, or other meals during the day. The cheese may also be consumed heated/melted. All participants will be provided vitamin D supplement of 40µg/day. The activity level is continuously recorded in the cheese-supplement – and activity record- form.

The participants meet for blood sampling in week 4 and 8 for measurements of Osteocalcin and Bone Turnover Markers (BTM). At week 8 week a clinical examination with measurement of vital signs and body weight will be performed. Additionally, blood samples will be taken for measurements of biochemical variables and vitamin K_2. The participants will be asked to fill in their diet for four days in the data application created for this study every 8 weeks. The diet registration will be performed and monitored.

6.2.3 Comparative phase 2:

All participants in both groups meet for the new investigation in week 16 and week 24, which is the last visit in the study. In both visits new clinical investigations will be performed with measurement of vital signs and body weight. During the last visit week 24, blood samples will be taken for measurements of Bone specific markers, vitamin K2 and biochemical variables. Sex hormones, muscle strength, Peak VO2 and DXA investigation of BMD,TBS, FFM and LM will be measured at in the same way as at day 0.

CTCAE version 4.0 will be used for registration of AE. In case of AE of grade 3 or 4 according to CTCAE occur for more than three days, the responsible investigator will act. The intake of cheese will be stopped and the XCS will be followed up until the symptoms disappear. The total duration from the last intake of the cheese to the disappearance of the symptoms will be recorded in days together with the treatment procedure. They will be reminded to fill in their dietary record.

6.2.4 Dietary monitoring:

The participants will fill in their dietary intake in an application at baseline and every 8th week of the study. During the last visit an Eating Disorder Examination Questionnaire- Short, derived from EDE-Q © Fairburn and Beglin[40], will be a part of the interview. The questions will be woven into the anamnestic interview and not as a questionnaire to ensure that the participants do not suffer from disordered eating.

6.3: Treatment administration of the study

6.3.1 Administration and doses

At baseline (week 0) the female and male strata will be provided a cheese sample for 4 weeks consumption of the type of cheese group they are allocated into. The female participants will be provided a sample resembling 75 grams cheese (5 slices) a day, and the male participants

will be provided 90 grams (6 slices) per day. The dose of Jarlsberg and Norvegia cheese is equal, and the dose is fixed due to OED from the former dose-finding study. After 4 weeks all participants will be provided new cheese samples of their study cheese. This will be repeated every 4 weeks till the end of the study at 24 as described previously. The dose of Jarlsberg and Norvegia cheese is equal.

Tabell II: Cheese Energy Contents per 100 grams

Energy Sources	Jarlsberg Cheese	Norvegia Cheese
Total Energy	1458 kJ (351 kcal)	1458 kJ(351kcal)
fat	27g	27 g
carbohydrates	0 g	0 g
protein	27g	27g
calcium	770mg	820mg
phosphate	550mg	590mg

6.3.2 Cheese supply, packing and storage.

The cheese will be delivered in 250-gram packages. The participants will receive the cheese by the investigator every 4 weeks. Both the Jarlsberg- and the Norvegia cheese used in the study will be given free of charge to the participating athletes.
Each package is labelled in accordance with the procedure for clinical trials. Additionally, the participants will be informed on how to perform the intake of the cheese and to store the cheese in a refrigerator at a temperature from 4° to10°C. Expiry date will be printed on the label.

Table III: Label on each package

Description	**For use only in clinical trial**
Trial substance	Type of cheese
Expire date	
Administration	Oral intake
Investigator	
Name of exporter	TINE A/S:
Phone number	+47 908 67088
Study	Comparative study in Active XCS
EudraCT number	2022-003264-24
Protocol id	XCS-Jarlsberg/IIA
Storage	Refrigerator between 4° to10°C

6.4: Stopping rule

In case of life-threatening AE or Serious Adverse Events (SAE) occurs, the cheese intake must stop, and the participant treated and followed up in accordance with GP-centre routines.

6.5: Procedures for Blood sampling and analysis.

Blood will be drawn via a cubital vein and separated into serum by centrifugation. Two ml serum will be sent Vitas for Osteocalcin analysis and 2 ml send for K2 analysis at the

biochemical laboratory of NMBU. 4 ml will be sent to the Hormone Laboratory of Aker University Hospital. The blood samples for measurements of the haematological and biochemical variables will be handled in accordance with the standard procedures at the laboratory of Norwegian School of Sports Sciences and sent to Fürst laboratory in Oslo for analysis.

6.6: Report of serious adverse effect (SAE)

The participant will be advised to contact the investigator if she/he suffers from severe AE or any other annoying conditions.
In the case of SAE, the investigator must complete the SAE form and send it to the health authorities with copy to TINE and the project manager Prof. Stig Larsen within 24 hours.

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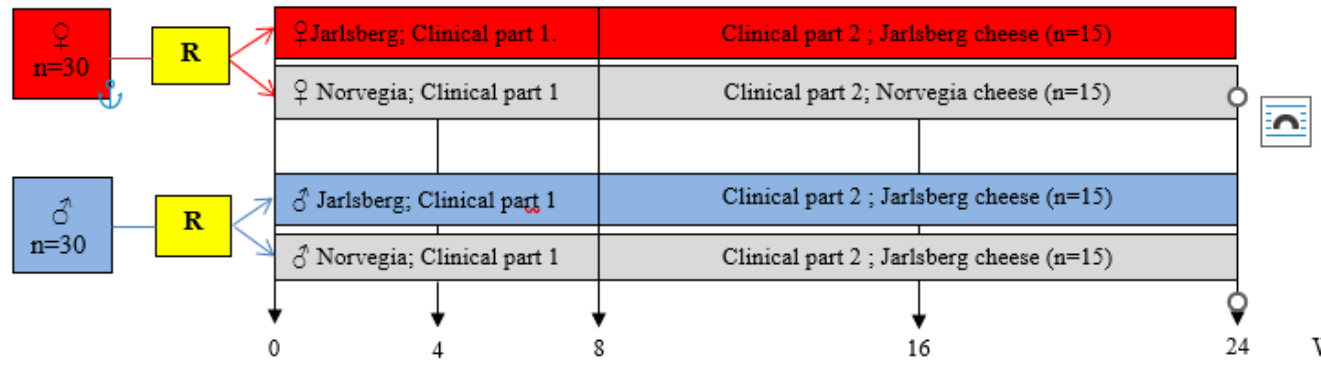
6.7: Time schedule

The duration of the study will be 24 weeks for each participant.

Recruitment period of XCS	01.01-31.05.23
Inclusion of the first participant	17th April 2023
Inclusion of the last participant	28th April 2023
Last participant finalized comparative study part	23rd June 2023
Last patient finalized follow-up part	14th Oct 2023

6.8: Flow diagram



Weight & Vital signs	X				X
Diet registration	X		X	X	X
Blood Sample (BS)	X		X	X	X
OC, K2; CTX, PINP, PTH	X	X	X	X	X
DXA measurement	X				X
Muscle strength &VO2	X				X
RMR	X				X
Adverse Events	X	X	X	X	X

VII: Project management and Monitoring

7.1: Project management

The study will be administered by a Steering committee headed by Dr Anne Cathrine Wist and consist of Dr Helge E Lundberg, Prof Jorunn Sundgot- Borgen, Associate Prof Therese Fostervold Mathisen, Prof. Emeritus Stig Larsen, Prof. Emeritus Helge Holo, Prof Emeritus Erik Fink Eriksen and Dr Tove Tveitan Borgen. The study will be monitored by a CRA from Meddoc. The data manager for this study will be Vivy Liang Larsen Meddoc Biometric Group, Norway and coordinated by Dr Anne Catherine Wist and Prof Stig Larsen. Dr Fagertun will be responsible of the Data Management and the Statistical Analysis and Prof Helge Holo for the laboratory analysis. The steering committee will have a joint responsible for publication of the results in international journals.

7.2. Quality assurance demands.

The validated data management (DM) system InCRF will be used for electronically collecting the data. The system selected is compliance with GCP guidelines and subject to 21 CFR (Code of federal regulations) FDA part 11 requirements. All the data created in this study will be entered at site, stored, and monitored electronically. The study case record forms (CRF) will be available at the DM-system and the data entered directly into the system at the site. Monitoring will be performed electronically and copies of the laboratory results and printed eCRF with Investigator's signature will be the source-documents.
In conducting the trial, TINE as the Sponsor accepts that the Ethics Committee or regulatory body may, at any time by appointment, conduct an audit of the study site, the laboratories conducting any clinical testing or the GMP manufacturing facilities.

7.3: Start-up and closing visit

The project manager and the clinical monitor will perform the start-up visit at the participating site. The visit will consist of a site inspection, information, instruction and handing the CRFs.
The project manager and the clinical monitor will perform the closing visit within one month after the last participant has finalized the study. All the trial material will be removed from the site.

7.4: Monitoring procedure

Essential demographic data will be documented with the participants' record notes as the source data and send to the monitor by e-mail after entering the InCRF-system. Source data will also include the date of written consent, times and dates of blood sampling and physical examinations.
It is the responsibility of the investigator to maintain accurate and up to date records of all clinical trial related activities, which should be legibly entered onto the CRFs provided. The CRFs should be made available in the event of a formal investigator site audit.
The site will be monitored seven times during the study. This includes both the start-up and the closing visits. Monitoring report will be sent to the project manager for every site inspection.

7.5. Curriculum vitae

All involved researchers participating in the study must show an updated CV documenting their expertise in the relevant clinical field. The CV must be signed and dated by the

physician and a copy must be attached to the protocol if required according to international rules. Another copy should be kept in The Trial Master File and a third copy in the Site File.

7.6. Site File

Meddoc will supply the investigator with a Site File. The Site File should contain all documents relevant for the study. The investigator is responsible to keep the Site File updated and secure that all required documents are present in the File. The Site File will be inspected during the monitor visits.

VII. Consideration

8.1: Consideration of steering committee

The study will be carried out according to the Helsinki declaration with latest amendments, Good Clinical Practice (GCP) and International Ethical Guidelines for Health-related Research Involving Humans (CIOMS guidelines). The participants are active cross-country skiers and will only be included in this clinical trial after approval of the trial by the regional Ethical Committee (REK) and after the XCS have received oral and written information and signed informed consent.
The product to be used in this trial is Jarlsberg cheese commercially available in Norway. To the best of our knowledge, no AE is reported except from person with intolerance of milk and milk product. It is known that vitamin K_2 passing a certain daily dose may increase and strengthen the bone tightness and may therefore have a prophylactic effect on bone fracture. This occurs frequently among active cross-country skiers. Jarlsberg cheese is shown to be one of the Norwegian produced cheeses with the highest level of vitamin K_2.
In a previously conducted dose-response study on cross country skiers we found the optimal efficacy dose (OED) to increase the osteocalcin levels. Our hypothesis is that this effect is caused by the fermentation of lactic acid to propionic acid by the Propionibacterium freudenreichii in the Jarlsberg Cheese, producing MK 9-4H K2 vitamer and DHNA. The first aim of this study is to compare the effects with the effect of Norvegia Cheese that does not contain MK 9-4H and DHNA to verify the hypothesis. Our second aim is to study the development of BTM, BMD, muscular strength and peak VO2 by the OED of Jarlsberg Cheese, to verify if the cheese could be recommended as prophylactical to prohibit LEA-related conditions.
All participants invited to this clinical trial are entitled to make their decision based on the fullest amount of information available at that time. In order to make the choice, they will be given a written document expressed in a clear concise language of their native tongue to consider. The document will tell potential participants about the aim of the study. Additionally, that blood sampling will be performed between five and eight times in connection with clinical examinations during the two study parts.

Summary: All the included volunteers will receive the daily intake of cheese and clinical examinations free of charge and receive a modest compensation for participation. All participants will be given oral and written information and have to give their written consent to participate in the study. To the best of our knowledge, this study fulfils the entire international requirement to an ethical controlled clinical trial.

8.2: Approval of the project

This study will be performed in Norway and the study protocol together and other requested information will be sent for approval by Regional Ethical Committee (REK). Inclusion of participants will not be started before the approval is received.
The database and storage will be in Norway and must be approved by the Data Register in Norway.

8.3: Informed consent

Before the start of the trial, the investigator will explain the confidentiality of participation in this research project, the objectives of the trial, the specific requirements for the participating

XCS, the trial design and the consequences of participation. Additionally, the investigator has to obtain written informed consent from the participants before inclusion in the study.

8.4: Protection of personal data

The monitor may know the identity of the participants during verification of the source data. However, the monitor has unconditional professional secrecy.
All participant-related material leaving the trial site will be anonymous so that the volunteer only can be identified by date of birth, initials and XCS study identification number. The investigator is responsible for keeping a list with the full names, their citizens' number, and corresponding study numbers according to the demands in GCP.
The participants will receive all the Jarlsberg cheese in the study for free. Additionally, they will get the clinical examination for free and receive a moderate economical compensation. In case they get extra transportation costs for the participation, this will be covered by the study.

IX. Data Management

9.1: Electronical Case Record Forms (eCRF)

The validated electronical data management system InCRF will be used for collecting the data. Mainly the data will be entered on eCRFs by the responsible investigator at stage. Prior to study start; a data entry instruction document will be made. In this study a copy of source data will be collected on a paper. Source data consist of both printouts from the laboratory. In case of printing CRF data from InCRF instead of using paper CRF, it is important that also these are stored. The printed CRF data need investigator's signature and date.

9.2: Study Database

The validated data management system InCRF will be used for collecting the CRF data. The system selected is compliant with GCP guidelines and subject to 21 CFR (Code of federal regulations) FDA part 11 requirements. The final database will be stored in the Statistical Analysis system (SAS ver. 9.4 or later).

9.3: Data handling

The DM will perform the data checks. Once all the errors or issues are corrected or resolved, the investigator shall sign all the eCRFs to acknowledge all the data in the database has reviewed and corrected. After all the eCRFs has electronically signed by the investigator, the Study Statistician will perform final SAS checks and proof reading of safety listing. Thereafter, the database can perform final database hard lock.
The database will be transformed to a labelled SAS database, which also will be locked to prevent all possible changes or additions. In this copy, the responsible statistician can make derivations but no corrections of the data. If corrections are needed, the main basic study database must be re-opened and corrected. The international procedure for such changes will be followed.

X: Discontinuation of treatment

A XCS may discontinue from the study at any time if in the view of the Investigator it is in the participant best interests. Alternatively, the XCS has the right to discontinue the consent and exit the study without prejudice regarding his/her future treatment or care.
If a XCS doesn't show up to an agreed visit the investigator should try to motivate the XCS to continue.

10.1: Discontinuation not related to the study question

XCS discontinues the study of administrative reasons or reasons documented not related to the trial treatment will be classified as "Drop out" and replaced by new XCS. The dropouts will be described specially in the statistical analysis.

10.2: Discontinuation related to the study question

XCS discontinues the study of reasons related or might be related to the trial treatment will be classified as "Withdrawal". These will not be replaced by new XCS but included in both the Per-Protocol (PP) and the ITT analysis using the procedure "Last observations carried forward" (LOCF).

XI Statistical Model

11.1 Presentation of results

Assumed continuously distributed variables expresses by mean values with 95% confidence intervals [37]. As an index of dispersion Standard Deviation (SD) will be used.
Categorized variables will be expressed in Contingency Tables [38.]

11.2 Statistical methods

The differences between the groups will be considered significant if the p-value is less or equal to 5 %. Comparison between groups regarding assumed continuously distributed variables will be performed by using analyses of covariance (ANCOVA) with a basic observation as covariance and repeated measurements [39]. Contingency table analyses will be used for comparison of discrete distributed variables.[38]. Correlation and regression analysis will be performed to estimate the connection in the development between variables.

11.3 Power analysis and sample size determination

With a significant level of 5%, a power of 90% and a clinically relevant difference in total osteocalcin increase of one-time SD between the two groups, at least 24 XCSs in each group must be included. By correcting for dropouts during the first part of the study, 30 XCS will be included in each group. Totally 60 XCSs will be recruited for participation[29].

XII: Operational Matter

12.1: Investigator's agreement

Before the start of the trial the investigator will confirm the agreement to participate in the trial by signing the Investigator's Agreement Form (Appendix IX).

12.2: Instructions

The investigators will give instructions by the data manager group and the clinical monitor supported by the project manager at the start-up visit and during the study.

12.3: Amendments to the protocol

Changes in the protocol can be required by the Ethical Committee, project manager or TINE. Changes must be written in amendments approved by the steering committee, investigator, Tina SA, and Ethical Committee. Amendments must be numbered and be together with the original protocol.
It is forbidden to add new parameters consisting of measurements on the patients in the study unless they are covered as amendments in the protocol or taken due to the health and safety of the patient.

12.4: Protocol deviations

Deviations from the protocol should be restricted as much as possible and will be fully recorded and justified. The Project manager will be informed as soon as possible of all protocol deviations.

12.5: Compliance monitoring

The Project manager and the Investigator will ensure that the site is suitable for the trial and that the participating XCS is well informed of the particulars of the trial. The Project manager and Investigators should check, protocol compliance, handling of the test articles and recording of data during the critical stages of the trial. A report is prepared of each visit and kept in the trial master file (TMF).

12.6: Responsibilities

The investigator will acknowledge the responsibilities and the agreement to participate in the trial by dating and signing the agreement form. The Project manager will verify that adequate arrangements have been made for the observations, measurements and recording of the data.

12.7: Confidentiality

The obtained data and results will be used by TINE for marketing purpose. The main study database will be stored in the product database of TINE.
The steering committee has the demand and the right to try the results published in an international medical journal. The draft of the manuscript must be presented for the sponsor for comments, discussion and final approve. The sponsor cannot stop the publication unless it is proved that publication of the results may destroy for marketing of the product.
The results from the study cannot be presented in any meeting or congresses without approval by the sponsor. The data obtained in this study must be handled confidentially by the member of the steering committee until the first registration of the product is performed.

XIII Amendment

13.1: Menstruation status (pkt. 5.9, 1.6 & 1.10)

To control the hormone blood sample results to female XCS, this group will be interviewed about their menstruation status. This includes menarche length, cycles day and use of hormonal contraceptives.

13.2: Inclusion criteria (pkt. 3,1 & 3.2 and 1.4)

3.1: Reference population. Active athletes of both sexes from 16 years of age representing sports with equal loading.
3.2: Study population. Active XCS from 16 years of age.
1.4: Population and sampling. The study population consists of active XCS of both genders past 16 years of age.

13.3: Cheese dose(pkt.1.6, 4.2 & 6.3.1)

4.2: The daily amount of cheese will be equal in the two cheese-groups with 5 slices (75g) for females and 6 slices (90g) for males.

13.4: Study procedure (pkt.1.6 & 5.2, 5.3 & 6.1, 6.2).

6.2: DXA investigation of BMD and TBS will be performed, and RMR measured together with muscle strength, LM and Peak VO_2 before starting the clinical part.

XIV References

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XV Appendix

14.1: Serious Adverse Event Form

14.2: The set of CRF's

14.3: Monitoring report form

14.4: CV investigators and study coordinators

14.5: Patient information