RESEARCH PROTOCOL

Final Version 1.5 7th. March 2023

Title

The effect on bone marker and body mineral density (BMD) of daily Jarlsberg Cheese intake in risk patient for Osteoporosis

Protocol number: PF Jarlsberg/IIIA
EudraCT number: 2022-003252-13
ClinicalTrial.gov: NCT

Sponsors:

TINE SA, Lakkegata 23, 0187 Oslo, Norway
FFL/JA: Norwegian Research Council

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- Skjetten Medical Centre (SMC), 2013 Skjetten, Norway
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List of Abbreviations

AE	Adverse Event
ANOVA	Analysis of Variance
ANCOVA	Analysis of Covariance
BALP	Bone specific alkaline phosphatase
BMD	Bone mineral density
BTM	Bone turnover marker
Ca.	Calcium
CRD	Clinically Relevant Difference
CRF	Case Report Form
cOC	Carboxylated Osteocalcin
CTCAE	Common Toxicity Criteria
CTX-1	Cross-linked C-telopeptide type I collagen
DXA	Bone Densitometry by X Ray
DHNA	1,4- Dihydroxy naphthoic Acid
DM	Data Management
FSH	Follicle stimulating hormone
GCP	Good Clinical Practice
GP	General Practitioner
ID	Identification
IEC	Independent Ethical XCS Committee
LH	Luteinising hormone
MR	Meddoc Research
MS	Muscle Strength
NMBU	Norges Miljø- og Biovitenskaplige Universitet
OED	Optimal Efficacy Dose
OP	Osteopeni
R_O	Osteocalcin ratio
PI	Principal Investigator
PINP	Procollagen type 1 N-terminal propeptide
PTH	Parathyroid hormone
QoL	Quality of Life
RSP	Response Surface Pathway design
SAE	Serious Adverse Event
SAS	Statistical Analysis Systems
SD	Standard Deviation
SHBG	Sexual hormone binding globulin
SOP	Standard Operating Procedure
OUH	Oslo University Hospital
ucOC	Under carboxylated Osteocalcin
UIO	University of Oslo

Distribution of Clinical trial Protocol

Complete Version	Date	Writer	Receivers	Internal review	Date released
Version 01	02.09.22	HEL	SL	No	No
Version 02	09.09.22	HEL	SL	No	No
Version 03	19.09.22	SL	HEL	No	No
Version 04	20.09.22	SL	HEL, ACW, HH, HF, VL, NT	Yes	No
Final version 1.0 (REK)	27.09.22	SL	HEL, ACW, HH, HF, VL, NT,TTB	Yes	27.09.22
Final version 1.1	11.10.22	SL	HEL, ACW, HH, HF, VL, NT, TTB	Yes	No
Final version 1.2 (REK)	17.01.23	SL	HEL, ACW, HH, HF, VL, NT, TTB, EFE	No	18.01.23
Final version 1.3 (REK)	01.02.23	SL	HEL, ACW, HH, HF, VL, NT, TTB, EFE, EL	No	01.02.23
Final version 1.4	07.02.23	SL	HEL, ACW, HH, HF, VL, NT, TTB, EFE, EL	Yes	No
Final version 1.5 (REK)	07.02.23	SL	HEL, ACW, HH, HF, VL, NT, TTB, EFE, EL, HDV, NS, SP, JK, KRG, SOL, QNU	Yes	07.03.23

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Study administration and performance

Prof. Emeritus Stig Larsen at Meddoc Research (MR) is the study manager and heading a study management group consisting of

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- Dr Anne Cathrine Whist Tine; Jarlsberg-3 coordinator
- Prof Emeritus Helge Holo (MR); Laboratory analyses
- Dr Helge Einar Lundberg (MR); Primary Investigator and clinical coordinator

This study is one of three clinical studies in the Jarlsberg-3 project. The scientific board of this project consists of

- Prof Emeritus Stig Larsen MR; Clinical research methodology and Statistic
- Prof Emeritus Erik Fink Eriksen; Treatment of Osteoporosis and measurements
- Dr Tove Tveitan Borgen; Rheumatology and patient in risk of Osteoporosis
- Dr Helge Einar Lundberg (MR); Primary Investigator

The following personnel at Meddoc Research is participating in the study.

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Seven investigators will participate and perform the clinical part of the study. These are:

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- Dr. Sosan Lotfi; Skjetten Medical Centre
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Contents

I: Synopsis

1.1: Title: The effect on bone marker and body mineral density (BMD) of daily Jarlsberg Cheese intake in risk patient for Osteoporosis.

1.2: Protocol number:

Protocol identification: PR-Jarlsberg/III_2022
Regional Ethical Committee number: 536927
EudraCT number: 2022-003252-13
ClinicalTrial.gov:

1.3: Aim

- To estimate the effect of daily optimal efficacy dose (OED) of Jarlsberg cheese on BMD and bone markers to patients with Osteopeni (OP).
- To compare daily intake of Calcium (Ca) and vitamin D with and without OED of Jarlsberg on BMD and bone markers to OP patients.
- To compare daily intake of Calcium (Ca) and vitamin D with and without OED of Jarlsberg on Time-to-Osteoporotic (TTO) treatment is recommended.

1.4: Study population: The study population consists of OP-patients of post-menopausal women and men above 50 years of age. OP patients are defined as patients with a T-score below 0.0 but larger than -2.5.

1.5: Trial treatment: The optimal daily intake of Jarlsberg cheese depending on gender, age, and physical activity.
Women in menopausal age have a daily OED Jarlsberg of 57 gram and men in the same age interval have an estimated OED of 65 gram.
Women in post-menopausal age have a daily OED Jarlsberg of XX gram and men in the same age interval have a daily OED of YY gram.
The cheese will be delivered in package of 250 gram with slices of 15.625gram. 57 gram represents 4 slices; 65 gram 4.5 slices; XX gram represents xx slices and YY represents yy slices. All the included patients will receive 20µg vitamin D and 1000 mg Ca. Half of the patients will receive vitamin D and Ca-tablets plus Jarlsberg cheese (Treatment group I) and other half only vitamin D and Ca-tablets (Treatment group II).

1.6: Design and randomization

1.6.1 Design:

The study will be performed as a randomized, single-blinded Norwegian multicentre trial with stratified semi-cross-over design with gender and participating General Practitioner (GP) site as stratification factors. 10 GP's from Viken county will participate.

1.6.2 Randomization:

Patients included in the study will be allocated to one of the two treatment groups by block randomization with random block size between 2 and 6.

1.7: Main variables: The main response variable will be the change in Bone Mineral Density (BMD) and recorded at baseline and every 16 weeks. The second main variables will be total Osteocalcin (tOC), carboxylated osteocalcin (cOC), the osteocalcin ratio R_O defined as the ratio between cOC and under-carboxylated osteocalcin (ucOC) [R_O = cOC / ucOC] and measured at start, week4, week 16, week 20, week 32 and week 48. Additionally, the bone markers cross-linked C-telopeptide type I collagen (CTX), procollagen type 1 N-terminal propeptide (PINP), bone specific ALP (BALB) and Parathyroid hormone (PTH) will be measured at the same visits. At baseline and every 16 weeks, the following variables will be measured: Vitamin K2 and the different vitamers MK-7, MK-8, MK-9, and MK-9(4H) used as explanatory variable; Diet registration as controlling variables and HbA1C, Lipids, biochemical variables together with "Quality-of-Life" (QoL) questionnaire as supporting variables. The Interleukins IL-1β, IL6, IL8, IL10 and the Cytokines TNF-α, NF-ƙβ (RANK-L), OPG and TGF-β will be measured initially and at the last visit in the study. The Common Terminology Criteria for Adverse Events version 4.0 (CTCAE) will be used for registration of Adverse Events (AE) and toxicity score at every investigation visit.

1.8: Study procedure

1.8.1 Screening and baseline

Participants, who fulfil the inclusion criteria, do not meet any of the exclusion criteria and willing to give informed consent to participate will be included in a screening period of one week. During this screening week, all the possible participants will receive 40µg vitamin D and 500 mg Ca tablets per day but asked not to eat Jarlsberg cheese.
DXA with BMD measurements will be taken at the start of the screening week. Patients with a BMD T-score ≤-1.0 will be included in the study starting with baseline measurements (Day 0). All demographic data, social factors, history of disease and vital signs will be recorded at baseline. The participating patients will be given a Diet-App on the web or mobile phone for registration of the daily food intake during four first consecutively days after baseline. Blood samples for measurements of Osteocalcin, CTX-1, PINP, BALP, PTH and vitamin K will be taken fastening in the morning. Additionally, blood samples for measuring biochemical variables, HbA1C, Lipids, Interleukins and Cytokines, will be recorded. The Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 used for measuring and classifying the tolerability and toxicity will be recorded as well. The patients will be given compliance & activity form and asked to write down the daily intake of treatment medicine, Jarlsberg cheese and hours of physical activities.

1.8.2 Clinical part

The patients will be allocated (1:1) to vitamin D + Ca-tablets ± Jarlsberg cheese. All the participants will get new delivery of vitamin D and Ca-tablets every four weeks. The patients allocated to Jarlsberg will additionally receive Jarlsberg cheese. The patients will receive a compliance & activity form and asked to fill in every day. All the patients meet for new visits at week4, week 16, and week 32. Patients allocated to only vitamin D + Ca tablets at baseline well additionally meet for visits at week 20 and week 48. At all these visits blood samples for

measurement of Osteocalcin, CTX-1, PINP, BALP and PTH will be drawn fasting in the morning, and concomitant medication and treatment compliance recorded.
The main visits will be performed every 16 weeks. New DXA screening will be performed. Patients obtaining a BMD T-score ≤ -2.5 will discontinue the study and switched to antiresorptive treatment plus daily OED of Jarlsberg cheese. The daily food intake during four consecutively days will be recorded on the Diet-App. Blood samples for measurements of Osteocalcin, CTX-1, PINP, BALB and PTH will be drawn fastening in the morning at every visits. Furthermore, blood samples for measuring and biochemical variables, HbA_1C and Lipids, will be recorded. The CTCAE will be recorded as well. The visit at Week 32 and Week 48 will be the last observation in the study for the patients initially allocated to Jarlsberg and controls, respectively. In addition to the mentioned variables blood samples for measurement of Interleukins and Cytokines will be performed. All the patients completed the study will be offered to continue the daily intake of Jarlsberg cheese. These patients will be followed up one year after the last visit with a new DXA and a blood sampling for measurements of the Osteocalcin level and the bone markers PINP, CTX and BALP.

1.9: Sample size: With a significance level of 5%, a power of 90% and a Clinically Relevant Difference (CRD) of one time the Standard Deviation in change of BMD, at least 24 patients in each group must finalize the study. With an expected drop-out rate of 25%, 30 patients in each group must be included.

1.10: Time schedule

A patient recruitment period of 12 months may be needed. The duration of each included patient will be between 32 and 48 weeks. The total duration of the study will be as follows:

Inclusion of the first patient	15-06-2023.
Inclusion of the last patient	15-06-2024.
The first patient finalized	15-01-2024.
The last patient finalized	15-12-2024.
Closing database	15-09-2025.
Finalized Clinical Study Report	15-10-2025.
Draft of manuscript for publication	15-12-2025.
One Year follow-up period	15.01.25 – 15.12.25.

1.11: Flow chart

ITEMS	Screening & Baseline	Weeks Comparison 4	16(0)	20 (4)	Switch 32(16)	48(32)	Follow-Up +1 Year
Inclusion and Exclusion criteria	x						
Oral & written informed consent	x						
Patient factors	x						
History of disease	x						
Diet registration	x		x		x	x	
Physical exam.	x		x		x	x	x
DXA-screening	x		x		x	x	x
Concomitant medication	x	x	x	x	x	x	x
Treatment compliance[1]		x	x	x	x	x	x
Blood sampling							
- Total Osteocalcin (tOC)	x	x	x	x	x	x	x
- Carboxylated Osteocalcin (cOC)	x	x	x	x	x	x	x
- Under carbox. Osteocalcin (ucOC)	x	x	x	x	x	x	x
- Collagen1 (CTX-1)	x	x	x	x	x	x	x
- Procollagen 1 (PINP)	x	x	x	x	x	x	x
- Bone specific ALP	x	x	x	x	x	x	x
- Parathyroid hormone (PTH)	x	x	x	x	x	x	x
- Vitamin K_1	x		x		x	x	
- MK-7	x		x		x	x	
- MK-8	x		x		x	x	
- MK-9	x		x		x	x	
- MK-9(4H)	x		x		x	x	
- Vitamin D	x		x		x	x	x
- Haemoglobin	x						
- Ferritin	x						
- Creatinine	x		x		x	x	
- HbA_1C	x		x		x	x	x
- Ca, Mg, Albumin	x		x		x	x	x
- Urea, Phosphate	x		x		x	x	x
- LDL-cholesterol	x		x		x	x	x
- HDL-cholesterol	x		x		x	x	x
- Interleukins /cytokines	x				x	x	
- TSH & FT4	x				x	x	
Adverse Events [CTCAE]	x	x	x	x	x	x	
End of treatment					x	x	

Red numbers indicate only the visits after switching to Jarlsberg in the control group.

[1] Treatment compliance will be performed every four weeks.

II: Introduction

2.1: Background

Bone loss remains a huge problem among the elderly. It is well established that dietary calcium and vitamin D are beneficial for skeletal health, but more recently research clearly demonstrates the importance of vitamin K and health claims stating the positive effects on the skeleton have been authorized by the European Food Safety Authority (EFSA). Dairy products are good calcium sources, important for bone formation. But cheese is also an important vitamin K, especially vitamin K2 source, indicating that cheese consumption may strengthen bones and reduce the risk of osteoporosis.

Recently, a Norwegian multicentre study comparing daily intake of Jarlsberg® and cheese without vitamin K2 has been performed[1]. 66 healthy female volunteers (HV) were recruited. By skewed randomization (3:2), 41 HV were allocated to daily intake of 57 g Jarlsberg® (J-group) and 25 to 50 g Camembert (C-group) in 6 weeks. After 6 weeks the C-group was switched to Jarlsberg®. The study duration was 12 weeks with clinical investigations every six weeks. The main variables were procollagen type 1 N-terminal propeptide (PINP), tOC, carboxylated osteocalcin (cOC) and the osteocalcin ratio (R_O) defined as the ratio between cOC and under-carboxylated osteocalcin. Serum cross-linked C-telopeptide type I collagen (CTX), vitamin K_2, lipids and clinical chemistry were used as secondary variables.

The study found that PINP, tOC, cOC, R_O and vitamin K_2 increased significantly ($p<0.01$) after 6 weeks in the J-group. PINP remained unchanged in the C-group. The other variables decreased slightly in the C-group but increased significantly ($p\leq0.05$) after switching to Jarlsberg®. No CTX-changes detected in neither of the groups. Serum lipids increased slightly in both groups. Switching to Jarlsberg, total cholesterol and LDL-cholesterol were significantly reduced ($p\leq0.05$). HbA1c, Ca^{++} and Mg^{++} were significantly reduced in the J-group, but unchanged in the C-group. Switching to Jarlsberg, HbA1c and Ca^{++} decreased significantly.

The optimal efficacy daily dose (OED) of Jarlsberg® cheese used in this study has previously been estimated in a study on fertile women.[2] A recently performed dose-response-study on post-menopausal female and male participants above the age of 50 estimated the OED of Jarlsberg cheese to be XX for women and YY for men. For participating fertile women in this new study, the Jarlsberg OED of 57 g/day estimated in the first dose-response study will be used[1]. For the other included patients, the OED estimated in the last dose-response study will be used. Previously only tOC, cOC and the Bone Turnover Markers (BMT) as PINP, CTX and Bone specific Alkaline Phosphatase (BALP) were recorded. In this study Body Mineral (BMD will be the main variable.

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[3]. Very low ORs have been associated with osteoporotic fractures[4].

Figure 1 shows the structure of common forms of vitamin K. Vitamin K_1 is produced in plants and found at high concentrations in leafy vegetables and is the dominating variant in the Western diet.

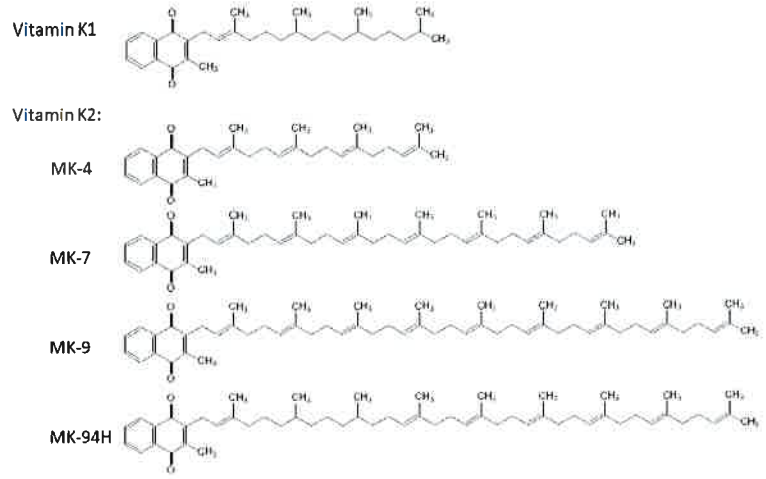

Figure 1: Structures of some vitamin K variants

Vitamin K2 (menaquinone, MK) is found in several variants (Fig 1). The short-chained MK-4 can be formed from vitamin K1 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 K2.
The long chained MKs have been found to have greater extra-hepatic activity than K1 and MK-4, possibly due to more efficient uptake and much longer serum half-life [5, 6]. Prospective cohort studies have demonstrated health benefits that can be attributed the intake of vitamin K2 but not K1, and the main contributor to the vitamin K2 is cheese containing MKs with long side chains [7, 8].

2.3: Jarlsberg Cheese

The dominating K_2 vitamer is MK-9 in most cheeses, but the amount varies considerably[9]. However, some cheeses also contain MK-9(4H). Jarlsberg cheese is rich in this compound[10]. This cheese is made with lactic acid bacteria producing MK-8 and MK-9 and *Propionibacterium freudenreichii (PF)* producing MK-9(4H).

Table I: Typical vitamin K_2 content of 100g Jarlsberg cheese

Component	
MK-8	3 µg
MK-9	13 µg
MK-9(4H)	75 µg

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 studies in healthy Norwegian women was performed with a 3-level between patient Response Surface Pathway design [1, 11]. These studies showed a significant increase of OC and cOC. Jarlsberg Cheese has been compared to Camembert cheese which does not contain any vitamin K2, and no similar effects on the Osteocalcin level was detected after Camembert consumption[2].

Table II: Nutritional content of 100g Jarlsberg® and Camembert Cheese

Nutritional content	Jarlsberg cheese	Camembert cheese
Energy	1458 kJ	1359 kJ
Fat	27 g	28 g
Saturated fatty acids	17 g	18 g
Carbohydrate	0 g	0 g
Sugars	0 g	0 g
Protein	27 g	19 g
Salt	1.1 g	1.5 g
Vitamin A	270 µg (34%)	280 µg (35%)
Riboflavin	0.32 mg (23%)	0.33 mg (24%)
Folic Acid	36 µg (18%)	51 µg (26%)
Vitamin B12	2.2 µg (88%)	1.7 µg (67%)
Calcium	770 mg (96%)	540 mg (68%)
Phosphorus	550 mg (79%)	390 mg (56%)
Zinc	4.2 mg (42%)	3.0 mg (30%)
Selena	12 µg (22%)	11 µg (20%)
Iodine	32 µg (21%)	45 µg (30%)

The mainly used prophylactic treatment against osteoporosis is still the combination of dietary calcium and vitamin D. This combination is shown to be beneficial for skeletal health, but not sufficient. To what extent will daily OED consumption of Jarlsberg cheese affect this effect?

2.4: Aims.

- To estimate the effect of daily optimal efficacy dose (OED) of Jarlsberg cheese on BMD and bone markers to patients with Borderline Osteoporosis (BO).
- To compare daily intake of Calcium (Ca) and vitamin D with and without OED of Jarlsberg on BMD and bone markers to BO patients.
- To compare daily intake of Calcium (Ca) and vitamin D with and without OED of Jarlsberg on Time-to-Osteoporotic (TTO) treatment is recommended.

III: Population and sampling

3.0: Study unit

The study unit is the participant.

3.1: Reference population

Men and women above the age of 55 years.

3.2: Study population

3.2.1: Inclusion criteria.

Osteopeni patients (OP) of both genders above 55 years of age. OPs are defined as patients with T-score below 0.0 but larger than -2.5.

3.2.2: Exclusion criteria.

Men and women who fulfils at least one of the following criteria will be excluded from participation in the study:

- Eating disorder
- Known gastrointestinal disorder.
- Abnormal liver or kidney function.
- Diabetes mellitus type I.
- Diabetes type II without sufficient control.
- Suffering from verified cancer.
- Under systemic treatment with corticosteroids or other immunosuppressive drugs the last 3 weeks before start of the trial treatment.
- Under active antiresorptive treatment or anabolic treatment.
- Participating in another clinical trial with pharmaceuticals the last six weeks before start of this trial treatment.
- Known milk product allergy.
- Not able to understand information.
- Do not want or not able to give written consent to participate in the study.

3.3: Recruitment of patients

Participants are recruited by reading an advertisement at the medical centre. If they are interested, they contact the responsible doctor by e-mail; hl@meddoc.no
Upon enquiry, this sends out current "Information for study participants" to the respective recruits. If, after reading this information, the person is interested and wants to participate, he/she will receive a time for the DXA measurement. If the results of this turn out to fall within the inclusion criteria, the participants make an appointment with one of the study doctors.
The recruitment letter is sent to the municipal superintendent in Lillestrøm municipality for distribution to the various doctors in the municipality. These can then look up the notice at their respective medical centres. Informed consent form must be obtained by medical

responsible physician in the study (Dr Helge Einar Lundberg) and not by someone with whom the patient has a treatment relationship. In case the patient wants to withdraw from the study, this can be stated to responsible physician classified as a neutral third party.

IV: Design and randomization

4.1: Study design

The study is performed as an open randomized semi crossover design with two strata. Sex is used as a stratification factor (Figure 1). The two strata are chosen because of different OED of cheese for women and men. Within each stratum the participants will be allocated either to vitamin D + Calcium alone (Control) or with daily OED of Jarlsberg cheese (Jarlsberg). The two groups will run parallelly in 16 weeks and BMD recorded by DXA. Patient with T-score<-2.5 classifies as Osteoporotic patients will be withdrawn from the study. At week 16, the patients in the control-group will be switched to additional daily OED of Jarlsberg cheese for 32 weeks. The patients randomised to Jarlsberg will continue with the same treatment for additionally 16 weeks.

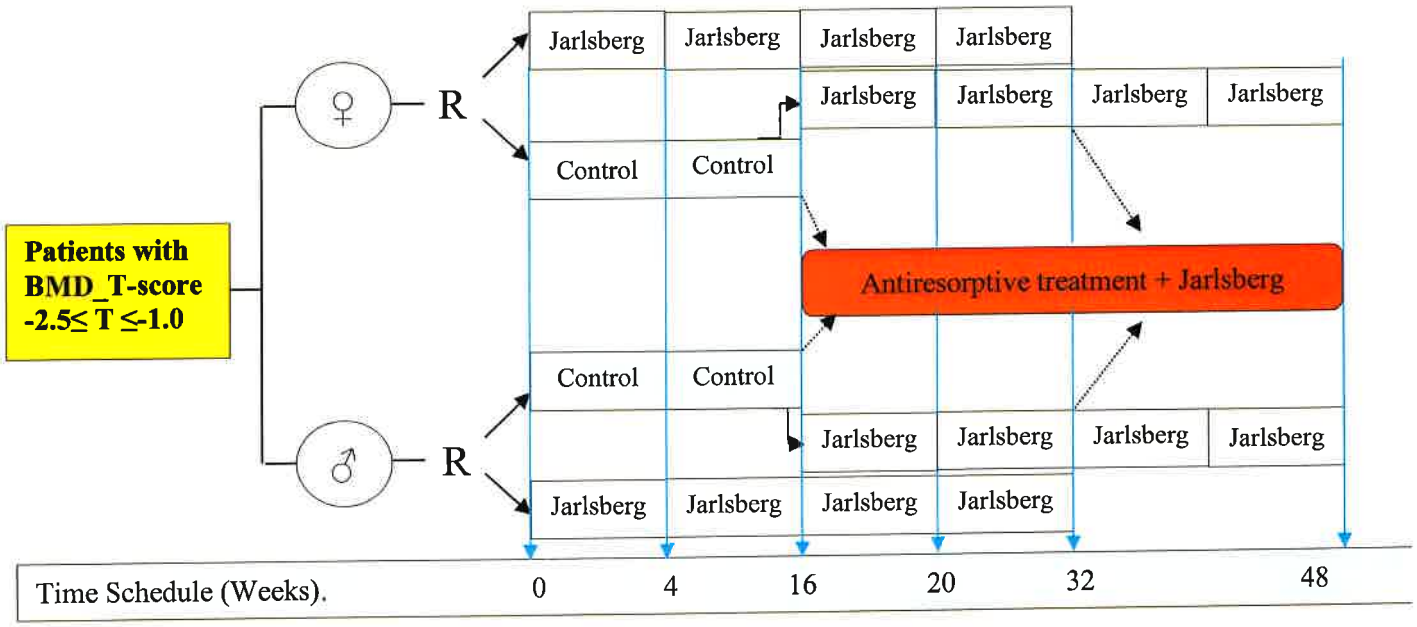

Figure 2: Study design with time schedule and variables to be recorded. Measurements given in red (x) is related only to the patients initially randomised to only vitamin D + Ca (control).

4.2: Randomization

All the participants will daily receive 20µg vitamin D and 1000 mg Ca. By randomization (1:1), half of the patient within each stratum will receive daily OED of Jarlsberg cheese. The allocation will be performed by block randomization with random block size between 2 and 10^{12}.

4.3: Identification of subjects

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

Digit 1: Indicate the investigator (01, 02, 03 up to 10)
Digit 2: Indicate the sex study arm (1=female, 2=male)
Digit 3&4: Indicate the number consecutively within each sex arm (01,02,03 etc.)

V: Evaluation

5.1: Bone mineral density (BMD)

DXA will be used as a surrogate measurement of BMD at baseline and every 16 weeks. BMD will be given in gram/cm^2 and use for estimating changes in total BMD, BMD L1-L4 and left and right neck of hip. The participants will be weighed in their underwear, and height will be measured with a fixed stadiometer. A dual energy X-ray absorptiometry performing the three-site scan i) lumbar area [L2-L4], ii) femoral neck, trochanter, and shaft [proximal femur], and iii) whole body compares the bone density with the bone density expected for a healthy adult of the same age, gender, and ethnicity as the patient. 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 classifies as follows[13]:

- 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

5.2: Osteocalcin and Bone turnover markers (BTM)

Blood samples for measurements of Osteocalcin, Procollagen (PINP), Collagen (CTX1), Bone specific Alkaline Phosphatase (BALP), Parathyroid hormone (PTH) will be taken at baseline and every 8 weeks[14, 15, 16, 17]. Osteocalcin will be recorded as serum Osteocalcin (tOC), carboxylated Osteocalcin (cOC), under carboxylated Osteocalcin (ucOC) and Osteocalcin ratio R_0= [cOC/ucOC].

5.3: Vitamin K

Blood samples for measurement of vitamin K will be taken at baseline and every 16 weeks. This includes vitamin K_1 and the different vitamers MK -7, -8, -9 and -9(4H).

5.4: Laboratory variables

Blood samples for measurements of haematological and clinical biochemical variables will be taken at baseline and every 16 weeks. The required variables to be measured are listed below.

5.4.1: Clinical chemistry

The following variables will be measured in serum:

Alkaline phosphatase (ALP)
Creatinine
Albumin
Urea
ALAT
Natrium
Calcium
Magnesium
Phosphate
Vitamin D
HbA_1C
Total Cholesterol

HDL cholesterol
LDL cholesterol

5.4.2: Haematology

The following haematological variables will be measured:
Haemoglobin (Hgb)
Erythrocytes
Ferritin
Thrombocytes
Leucocytes

5.4.2: Interleukins and Cytokines

Blood samples for measurements Interleukins (IL) and Cytokines will be taken at baseline and the last visit in the study. The chosen interleukins are IL-1β, IL-6, IL8, IL10 and the cytokine TNF-α, NF-ƙβ (RANK-L), OPG and TGF-β.

5.5: Diet registration

Diet registration will be performed by the participants during the screening period and every 16 weeks. The daily intake of all food will be recorded daily in four consecutive days after inclusion in the screening period and after every 16-week visits. 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.

5.6: Adverse Events (AE)

5.6.1: 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[21]. 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)

5.6.2: 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.

5.6.3: 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.

5.7 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. The patients will daily recording the intake of study treatment and physical activity on a compliance & activity form.

VI: Study procedure

6.1: Definitions

Important definitions are:
Osteocalcin Ratio (R_o): cOC / uOC.

6.2 Clinical procedure

6.2.1: Screening phase

The recruited patients fulfilling the inclusion and avoided the exclusion criteria will be registered and asked to participate in the study. The patients who are willing to sign the informed consent form will be enrolled in the study. The first part of the study is a screening phase of one week duration. The participants will undergo a clinical investigation and an appointment for the starting visit in the study one week later. In the meantime, a dietary record, activity record and the first DXA for measurement of BMD will be performed. The dietary measurements will be supervised by the monitor. All the participants will be instructed not to change their usual intake of food during the study but change their usual consumed cheese with the study cheese. During this screening period an initial clinical examination will be performed. At this baseline visit, all demographic data, social factors, history of disease and vital signs will be recorded. Blood samples will be collected for measurements of Osteocalcin, vitamin K2 and DHNA; Hematological and biochemical variables including HbA1C and Lipids, together with the BTMs and Interleukins. At the baseline visit all the patients will receive 20µg vitamin D and 1000 mg Ca for 4 weeks. Additionally, half of the patients will randomly be allocated to daily OED of Jarlsberg cheese and receives cheese for the next 4 weeks. All the patients will receive a registration form for daily intake of Jarlsberg cheese and vitamin D + Ca. 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. New supply of vitamin D + Ca and cheese will be handed over to the patients by the one responsible person for the Meddoc team together with a new registration form.

6.2.2: Comparative phase

All the participants meet for clinical investigations at week 4 and week 16 in the comparative phase of the study. At week 4, blood samples will be taken for measurements of Osteocalcin and the BTMs. Additionally, concomitant medication, adverse events (AE) and treatment compliance will be recorded. Cheese and vitamin D + Ca for the next 4 weeks will be delivered. A new delivery will be handed over by a representative from the Meddoc team at week 8 and week 12 in the study. The last clinical investigation in this comparative phase will be performed at week 16. The physical examination will be equal with the one performed in the screening phase. The patients must perform a new diet registration during the following 4 days and undergo the second DXA investigation for measurements of BMD. Additionally, the patients will record their change in concomitant medication. Blood samples will be collected for measurements of Osteocalcin, vitamin K2, BTMs, HbA1C, Lipids and other hematological and biochemical variables. Patients with a T-score < -2.5 based on the DXA investigation will be classified as Osteoporotic and withdrawn from the study. If possible and

in accordance with the responsible investigator, the patient will be given Antiresorptive treatment in addition to OED of Jarlsberg cheese and vitamin D + Ca.
The patients randomized to only vitamin D + Ca (Controls) at baseline will now receive OED of Jarlsberg cheese equal with the other patients and continue in the follow-up phase of the study. Cheese for the next 4 weeks will be delivered together with compliance & activity form.

6.2.3: Follow-up phase

The patients allocated to Vitamin D + Ca at baseline will meet for a new investigation after 4 weeks which is week 20 in the study. Blood samples will be taken for measurements of Osteocalcin and the BTMs. Additionally, concomitant medication, AE and treatment compliance will be recorded. Cheese for the next 4 weeks will be delivered. A new delivery will be handed over by a representative from the Meddoc team every week. At every delivery the patients receive a new treatment & activity form and asked to perform daily registrations. The last visit in the follow-up part will be at week 32 for the patients randomized to OED of Jarlsberg cheese at baseline. The physical examination will be equal with the one previously performed. The patients must perform a new diet registration during the following 4 days and undergo the third DXA investigation for measurements of BMD. Additionally, the patients will record their change in concomitant medication. Blood samples will be collected for measurements of Osteocalcin, vitamin K2, BTMs, HbA1C, Lipids, Interleukins, cytokines, and other hematological and biochemical variables. Patients with a T-score < -2.5 based on the DXA investigation will be classified as Osteoporotic and withdrawn from the study. If possible and in accordance with the responsible investigator, the patient will be given Antiresorptive treatment together with OED of Jarlsberg cheese and vitamin D + Ca.
The patients allocated to vitamin D + Ca only at baseline will meet for a new visit at week 32 and the last visit in the follow-up part at weeks 48. These investigations will be equal to those performed at week 16 and 32 for the Jarlsberg group.

6.2.4: End of study

The end of the study will be Week 32 for the patients allocated to Jarlsberg cheese at baseline and Week 48 for those allocated to vitamin D + Ca at baseline. At the end of the study, the "End-of-treatment" Case Record Form (CRF) must be filled in by the investigator.

6.2.5: One year follow-up

The patient finalizing the study will be asked if they want to continue with daily OED of Jarlsberg cheese. They will receive the cheese free of charge but must meet for a new investigation one year later. This investigation will consist of a physical examination, and a DXA measurement. A new blood sample will be taken for measurement of Osteocalcin, BTMs, vitamin K_2, lipids and HbA_1C. Additionally, the number of bone fractures during the last year will be recorded.

6.3: Treatment administration of the study

6.3.1: Daily doses

All the patients will receive 20µg vitamin D and 1000 mg Calcium (Ca) tablets daily during the study. The daily Optimal Efficacy Dose (OED) of Jarlsberg differs between genders and menopausal stage. For fertile women the daily OED of Jarlsberg will be 57 g. For men passed the age of 50 years it is XX g and for post-menopausal women it is YY g.

Tabell III: Cheese Energy Contents per 100 grams

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

6.3.2: The supply of Cheese, vitamin D + Ca

The patient will receive the cheese and vitamin D + Ca at the visits every 8 weeks during the study. However, the patients needed new delivery every four weeks. These deliveries in between will be performed by a study nurse in the Meddoc team who brings the new delivery home to the patients and collecting the compliance & activity form.

6.3.2: Packing and storage.

The cheese will be delivered in 250-gram packages from Tine. The Jarlsberg cheese and the supplements used in the study will be given free of charge to the patients.
Each package is labeled 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 IIV: Label on each package

Description	**For use only in clinical trial**
Trial substance	Jarlsberg® Original
Expire date	
Administration	Oral intake
Investigator	
Name of exporter	TINE A/S:
Phone number	+47 908 67088
Study	Comparative study in risk patient for Osteoporosis
EudraCT number	
Protocol id	PF-Jarlsberg/IIIA
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 patient treated and followed up in accordance with GP-center routines.

6.5: Procedures for Blood sampling and analysis.

Blood will be drawn from the fasting patients via a cubital vein and separated into serum by centrifugation. Two ml serum will be sending Vitas for Osteocalcin analysis and 2 ml send for K2 analysis at the biochemical laboratory of NMBU. The blood samples for measurements of the hematological and biochemical variables will be handled in accordance with the standard procedures at the GP-center and send to Fürst laboratory and The Hormone laboratory of Oslo University Hospital in Oslo for analysis.

6.6: Report of serious adverse effect (SAE)

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

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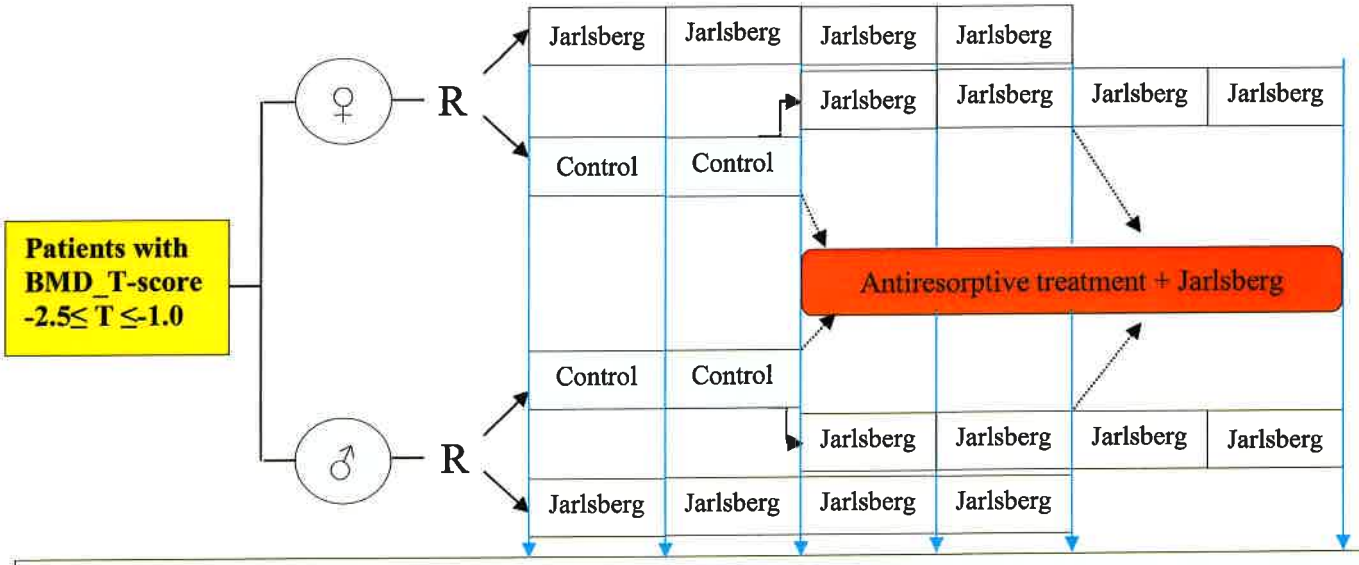
6.7: Time schedule

A patient recruitment period of 12 months may be needed. The duration of each included patient will be between 32 and 48 weeks. The total duration of the study will be as follows:

Inclusion of the first patient	15-06-2023.
Inclusion of the last patient	15-06-2024.
The first patient finalized	15-01-2024.
The last patient finalized	15-12-2024.
Closing database	15-09-2025.
Finalized Clinical Study Report	15-10-2025.
Draft of manuscript for publication	15-12-2025.
One Year follow-up period	15.01.25 – 15.12.25.



Time Schedule (Weeks).	0	4	16	20	32	48
Clinical investigation	x		x		x	x
Specified biochemistry.	x		x		x	x
DXA	x		x		x	x
Diet registration	x		x		x	x
Lipids	x		x		x	x
CTCAE	x	x	x	x	x	x
Osteocalcin & Bone markers	x	x	x	x	x	x
K2 vitamers	x		x		x	x
End-of-study					x	x

x) is related to the patients initially allocated to only Calcium + vitamin D (Control group)

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 Emeritus Stig Larsen, and Prof Helge Holo. 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 Cathrine Wist and Prof. Emeritus 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.

7.7. Fees

Neither the project manager nor study employees will receive special remuneration for their participation in the study. They are fast paid from their respective institutions. In contrast, the study budget earmarks NOK 20,000 for the scientific panel. The participating doctors, including laboratory staff who carry out the clinical work, receive NOK 11 000 per patient. The professionals named in the protocol will be remunerated through participation in all publication and presentation of results from studies. The participating patients will not receive any fee for participation, but the study will cover extra travel expenses in connection with visiting the doctor and other examinations in the study.

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 risk patient for Osteoporosis and will only be included in this clinical trial after approval of the trial by the regional Ethical Committee (REK) and the patients 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 density and may therefore have a prophylactic effect on bone fracture. 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 healthy fertile women we found the optimal efficacy dose (OED) to increase the osteocalcin levels[1, 2, 11]. 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 commonly used prophylactic treatment of vitamin D + calcium to verify the hypothesis. Our second aim is to study the development of BTM and BMD by the OED of Jarlsberg Cheese and to verify if the cheese could be recommended as prophylactical treatment to osteoporosis.

All participants invited to this clinical trial are entitled to make their decision based on the fullest amount of information available at that time. 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 seven times in connection with clinical examinations during the two study parts.

Summary: All the included patients will receive the daily intake of cheese, supplement, and clinical examinations free of charge. All participants will be given oral and written information and must 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 Norwegian Centre of Research Data" (NSD).

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 patients, the trial design, and the consequences of participation. Additionally, the investigator must 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 patients only can be identified by date of birth, initials, and 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 + supplements and the clinical examination for free.
In accordance with Norwegian and international roles, the data from this study will be stored in at least 15 years.

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

10.1: Discontinuation not related to the study question

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

10.2: Discontinuation related to the study question

If patient discontinue 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 participants 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 [22]. As an index of dispersion Standard Deviation (SD) will be used.
Categorized variables will be expressed in Contingency Tables [23]

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 [24]. Contingency table analyses will be used for comparison of discrete distributed variables.[23]. 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 significance level of 5 %, detection power level of 95 %, and a clinical relevance difference defined as 1xSD of BMD, the number of patients included in each group will be at least 24 patients in each group finalizing the study [26, 27].
Based on our previous study on cross country skier (XCS), the Coefficient of Variance (CV) in total BMD was found to be 0.07 with SD = 0.09 g/cm^2 for both sexes [28]. It is reasonable to assume that the variation in BMD among elderly patients is below the dispersion in young active XCS. Daily intake of Jarlsberg cheese during 12 weeks in XCS detected a significant increase in BMD of 0.1 g/cm^2. Vitamin D + Ca is recommended to reduces bone loss, but it is reasonable to assume none or limited increase in BMD. From these results it is reasonable to define a clinically relevant difference between the group of 1xSD=0.09 g/cm^2. To correct for the multicentre situation and drop-out rate, 30 patients in each group must be included [27].

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, TINE 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 participants are 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 References

13.1: References

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XIV 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