RESEARCH PROTOCOL

Final version 4 12th February 2020

Title

Effect of Jarlsberg cheese compared to cheese without vitamin K2 (Camembert) regarding increased Osteocalcin level in healthy women

Protocol number: HV-Jarlsberg/III
EudraCT number: 2019-004593-26
ClinicalTrial.gov: NCT04189796

Sponsor

TINE SA, Lakkegata 23, 0187 Oslo, Norway

Administration

Project Manager: Professor Stig Larsen
Norwegian University of Life Sciences, Oslo Norway

Project coordinator: Dr. Trond Holand
Norwegian University of Life Sciences, Oslo Norway

Research protocol HV-Jarlsberg/III

0: Preface

The following steering committee will administer the study:

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Prof. Stig Larsen has written the trial protocol with support from the steering committee. The statistical analysis will be performed by Hans Fagertun and together with Stig Larsen. They are responsible for writing the integrated clinical and statistical report.
The result aims to be published in an international medical journal and the manuscript prepared by the steering committee. The study will be monitored by Natharat Thiendilokkul (BSc) and Data Management by Vivy Liang Larsen (MSc) from the Meddoc Biometric group in Norway.

Research protocol HV-Jarlsberg/III

0.1: Signature Sheet

Protocol Authorized:

Stig Larsen[1] and Helge Holo[2]

Qualifications:

1) Professor: Faculty of Veterinary Medicine; NMBU
2) Professor: Faculty of Biotechnology and Food Science; NMBU

Signature

1:……..Date:……

Signature

2:……..Date:……

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0.2: Contact names and addresses

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0.3: List of Abbreviations

Abbreviation	Explanation
AE	Adverse Event
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
BSC	Bachelor of Science
CPK	Creatinine Phosphokinase
CRF	Case Report Form
CRP	C-Reactive Protein
CTCAE	Common Terminology Criteria for Adverse Events
CV	Curriculum Vita
DM	Data Management
FNB	Food and Nutrition Board
GCP	Good Clinical Practice
IP	Investigational Product
ITT	Intention to treat
LDH	Lactate Dehydrogenase
LOCF	Last Observation Carried Forward
MEDD	Minimum efficacy daily dose
MSC	Master of Science
OR	Osteocalcin Ratio
PP	Per Protocol
REK	Regional Ethical Committee
RSP	Response Surface Pathway
SAE	Serious Adverse Event
SD	Standard Deviation
SOC	System Organ Class

Research protocol HV-Jarlsberg/III

0.4: Distribution of Clinical trial Protocols

Complete Version	Date	Writer	Receivers	Internal review	External review
Version 01	22.03.2019	SL	HF,VLL,NT,TH,HH,HEL	Yes	No
Version 02	01.04.2019	SL	HF,VLL,NT,TH,HH,HEL	Yes	No
Version 03	05.04.2019	SL	HF,VLL,NT,TH,HH,HEL	Yes	No
Version 04	15.08.2019	SL	HF,VLL,NT,TH,HH,HEL	Yes	No
Version 05	06.09.2019	SL	HF,VLL,NT,TH,HH,HEL	Yes	No
Version 06	11.09.2019	SL	HF,VLL,NT,TH,HH,HEL	Yes	No
Final version 1	24.09.2019	SL	HF,VLL,NT,TH,HH,HEL	Yes	No
Final version 2	25.11.2019	SL	HF,VLL,NT,TH,HH,HEL,TA,KH,ZS,SI, AS,RC	Yes	Yes
Final version 3	10.12.2019	SL	HF,VLL,NT,TH,HH,HEL,TA,KH,ZS,SI, AS,RC,MG	Yes	Yes
Final version 3	12.02.2020	SL	HF,VLL,NT,TH,HH,HEL,TA,KH,ZS,SI, AS,RC,MG	Yes	Yes

SL = Stig Larsen
HF = Hans Fagertun
VLL= Vivy Liang Larsen
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TH = Trond Holand
HH = Helge Holo
HEL = Helge Einar Lundberg
TA = Torunn L Austlid
KH = Kathrine Hovland
ZS = Zohaib Sarwar
SI = Sapna Iqbal
AS = André Shukla
RC = Rahul Chhura
MG= Morten Glasø

0.5: Participating Institutions

- Faculty of Biotechnology and Food Science, Norwegian University of Life Sciences, Ås, Norway
- Faculty of Veterinary medicine, Norwegian University of Life Science, Oslo, Norway
- Skjetten Medical GP-centre; Skjetten, Norway
- Stallbakken Medical GP-center; Rælingen, Norway
- Eidsvold Medical GP-center; Eidsvold, Norway
- Meddoc AS, Skjetten Norway

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I: Protocol Synopsis

1.1: Project title

Effect of Jarlsberg cheese compared to Camembert cheese without vitamin K_2 regarding increased Osteocalcin levels in healthy women

1.2: Protocol numbers

Protocol number: HV-Jarlsberg/III
Regional Ethical Committee number:
EudraCT number: 2019-004593-26
ClinicalTrial.gov: NCT04189796

1.3: Objectives

The study objective consists of the following three aims:

1. To compare the effect of daily intake of Jarlsberg cheese and cheese without content of Vitamin K2 (Camembert) in change of the Osteocalcin level in healthy women after 6 weeks.
2. To estimate the long-term increase of the osteocalcin level, change in the lipid pattern and the vital signs caused by optimized daily intake of Jarlsberg cheese.
3. To verify the estimated maintaining dose of Jarlsberg cheese related to stabilized osteocalcin level

1.4: Population and sampling

The study population consists of Healthy Voluntary (HV) women between 20 years and pre-menopausal age.

1.5: Design and randomization

The study will be performed as a randomized Norwegian multicenter study with a semi-cross over design in which the participants randomized to Camembert cheese will be switched to Jarlsberg cheese after 6 weeks.

1.6: Study procedure

The recruited HV women fulfilled the inclusion without the exclusion criteria for the study will undergo a screening clinical investigation. The participants will be asked to avoid use of other cheese than the one allocated to in the study but eat as usual. One week later, the first clinical investigation in the study will take place including blood sampling and measurement of vital signs. The HV women verified to fulfil the criteria for participation and signed the informed consent form will be included in the study. During this first clinical investigation in the study denoted as Day 0, the participants receive a study identification number. The HV will randomly be allocated to either daily intake of Jarlsberg cheese or Camembert cheese without content of vitamin K2. The daily intake of Jarlsberg cheese will be 57g/day and 50g/day Camembert cheese. The two cheese doses are nearly equal with regard to total energy, protein carbohydrate and fat. The trial cheese can be consumed with other food at breakfast, lunch or other meals during the day.

The participants meet for new clinical investigations every third week with measurement of vital signs and blood sampling. Osteocalcin and vitamin K will be analysed every third week whereas the haematological and biochemical analysis will be performed every six week. The HVs allocated to Camembert cheese will after 6 weeks be switched to daily intake of Jarlsberg cheese in additional 6 weeks with clinical investigations after 3 and 6 weeks. The participants performed the 6 weeks of daily intake of Jarlsberg cheese either by randomization or switching to Jarlsberg cheese will either be offered participation in the cheese de-escalation study (HV-Jarlsberg/IB) or an extended study of 6 weeks with unchanged Jarlsberg cheese dose. The first 12 HVs finalized 6 weeks with daily intake of Jarlsberg cheese obtaining an increase in the osteocalcin level from baseline ≥10% will be allocated to the de-escalation study HV-Jarlsberg/IB (separate protocol). The HVs included in the extended part of this study will receive an unchanged daily dose of Jarlsberg cheese for additional 6 weeks with clinical investigation every third week. The HVs switched to Jarlsberg cheese may be offered participation in a study part aiming to verify the maintaining dose obtained in HV-Jarlsberg/IB study. The duration of this part will be 6 weeks with clinical investigation every third week.

1.7: Main variables

The main variable in this study will be osteocalcin measured in blood serum. Additionally, carboxylated and under carboxylated Osteocalcin and the ratio OR = [Carboxylated / Under Carboxylated] osteocalcin in serum will be central together the K2 variants MK-7, 8, 9, 9(4H) and vitamin K1. Triglyceride, LDL- and HDL cholesterol, vitamin D and vital signs will be secondary variables. As safety variables, haematological- and biochemical variables and adverse events (AE) will be recorded at each visit.

1.8: Sample size

With a significant level of 5%, a power of 90% and a clinical relevant difference in total osteocalcin increase of one-time SD between the two groups, at least 24 HVs in each group have to be included. By correcting for the number of participating General Practitioners (GP) and drop-outs during the first part of the study, 32 HVs will be included in each group. Totally 64 HVs will be recruited from the eight participating GP sites.

1.9: Study duration

The duration of the first comparative part of the study will vary from 6 to 12 weeks depending on allocation to Jarlsberg cheese or the alternative cheese. The duration of the expending Jarlsberg part or the maintaining dose part will be 6 weeks. The total duration of the study will be maximum 18 weeks:

Inclusion of the first participant	13th January	2020
Inclusion of the last participant	24th February	2020
Last participant finalized comparative study part	14th April	2020
Last patient finalized extended part	6th July	2020
Final statistical reports	18th September	2020

Research protocol HV-Jarlsberg/III

1.10: Flow Chart

Trial procedure	Screening	Baseline & Day0	Comparative study part				Extended	
			Week 3	Week 6	Week 9	Week 12	Week 15	Week 18
Jarlsberg cheese group		x	x	x	x	x		
Camembert cheese group		x	x	x				
Switched to Jarlsberg cheese					x	x	x	x
Inclusion / Exclusion criteria.	x							
Patient factors	x							
history of disease	x							
Concomitant treatment	x	x	x	x	x	x	x	x
Vital signs								
-Systolic BP		x	x	x	x	x	x	x
-Diastolic BP		x	x	x	x	x	x	x
-Pulse rate		x	x	x	x	x	x	x
Blood sampling								
-Osteocalcin		x	x	x	x	x		x
-Vitamin K_1		x	x	x	x	x		x
-Vitamin K_2		x	x	x	x	x		x
-Vitamin D		x		x		x		x
-Triglyceride		x		x		x		x
-LDL Cholesterol		x		x		x		x
-HDL Cholesterol		x		x		x		x
-Hematology		x		x		x		x
-Biochemical		x		x		x		x
Adverse Events [CTCAE]		x	x	x	x	x	x	x
Cheese compliance			x	x	x	x	x	x

x) Indicate visits in the Camembert group after switching to Jarlsberg

Research protocol HV-Jarlsberg/III

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. The effect of eating vitamin K2 rich cheese on bone health has not been studied.

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 [1]. Very low ORs have been associated with osteoporotic fractures [2].
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.

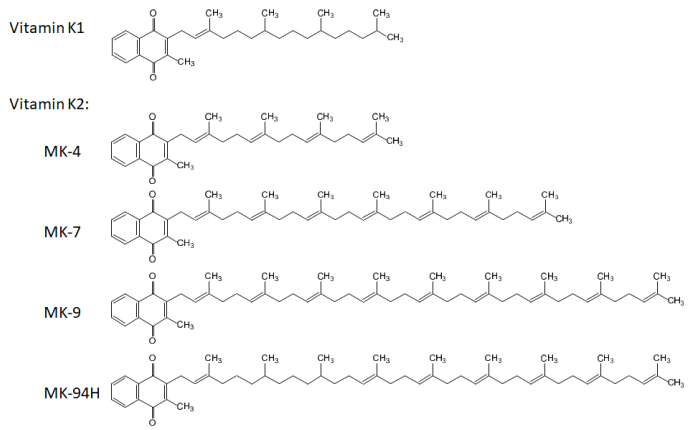

Figure 1: Structures of some vitamin K variants

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 variants 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 [3, 4].
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 [5, 6].

2.3: Jarlsberg Cheese

The dominating vitamin K2 variant is MK-9 in most cheeses, but the amount varies considerably [7]. However, some cheeses also contain MK-9(4H). Jarlsberg cheese in particular is rich in this compound [8]. This cheese is made with lactic acid bacteria producing MK-8 and MK-9 and *Propionibacterium freudenreichii* producing MK-9(4H).
Although vitamin K2 related health benefits have been associated with cheese, the effects of cheese consumption on bone health has never been studied in intervention studies. Because of its high vitamin K2 content Jarlsberg cheese is well suited for such a study.

Table I: Typical vitamin K2 content of 100g Jarlsberg cheese

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

Recently, a dose-response study in healthy Norwegian women was performed with a 3-level between patient Response Surface Pathway design [9, 10, 11]. The study was performed in 19 women with daily intake of Jarlsberg cheese during a period of 5 weeks [12].

2.4: Comparative Cheese

As comparable cheese, Camembert in a daily dose of 50 g was chosen.

Table II: Nutritional content of 100g of the comparative 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%)

Camembert cheese is without vitamin K and the daily comparative dose of 50 gram Camembert represents 680 kJ and 14.0 gram fat. The daily dose with Jarlsberg cheese is 57 gram, representing 729 kJ and 15.4 gram fat.

2.5: Objectives

The study objective consists of the following three aims:

1. To compare the effect of daily intake of Jarlsberg cheese and cheese without content of Vitamin K_2 (Camembert) in change of the Osteocalcin level in healthy women after 6 weeks.
2. To estimate the long-term increase of the osteocalcin level, change in the lipid pattern and the vital signs caused by optimized daily intake of Jarlsberg cheese.
3. To verify the estimated maintaining dose of Jarlsberg cheese related to stabilized osteocalcin level

III: Population and sampling

3.1: Reference population

The reference population consists of heathy women (HV) in pre-menopausal age.

3.2: Study population

3.2.1: Inclusion criteria

HV from 20 years of age and within pre-menopausal age

3.2.2: Exclusion criteria.

Health volunteers fulfil at least one of the following criteria will be excluded from participation in the study:

- Pregnant women
- 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.
- LDL-cholesterol > 3 mmol/L or Triglyceride > 2 mmol/l
- Participating in another clinical trial with pharmaceuticals the last six weeks before start of this trial treatment.
- Lactose intolerance or 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

The volunteers will be recruited by the participating General Practitioner (GP) centers. HV fulfils the inclusion and none of the exclusion criteria will be asked to participate. The HV has to give written consent for participation.

IV: Design

4.1: Project design

This project consists of one comparative study HV-Jarlsberg/III and one dose de-escalation study HV-Jarlsberg/IB.
The HVs to be included in the de-escalation study will be recruited from HV-Jarlsberg/III after finalized 6 weeks with daily use of Jarlsberg cheese

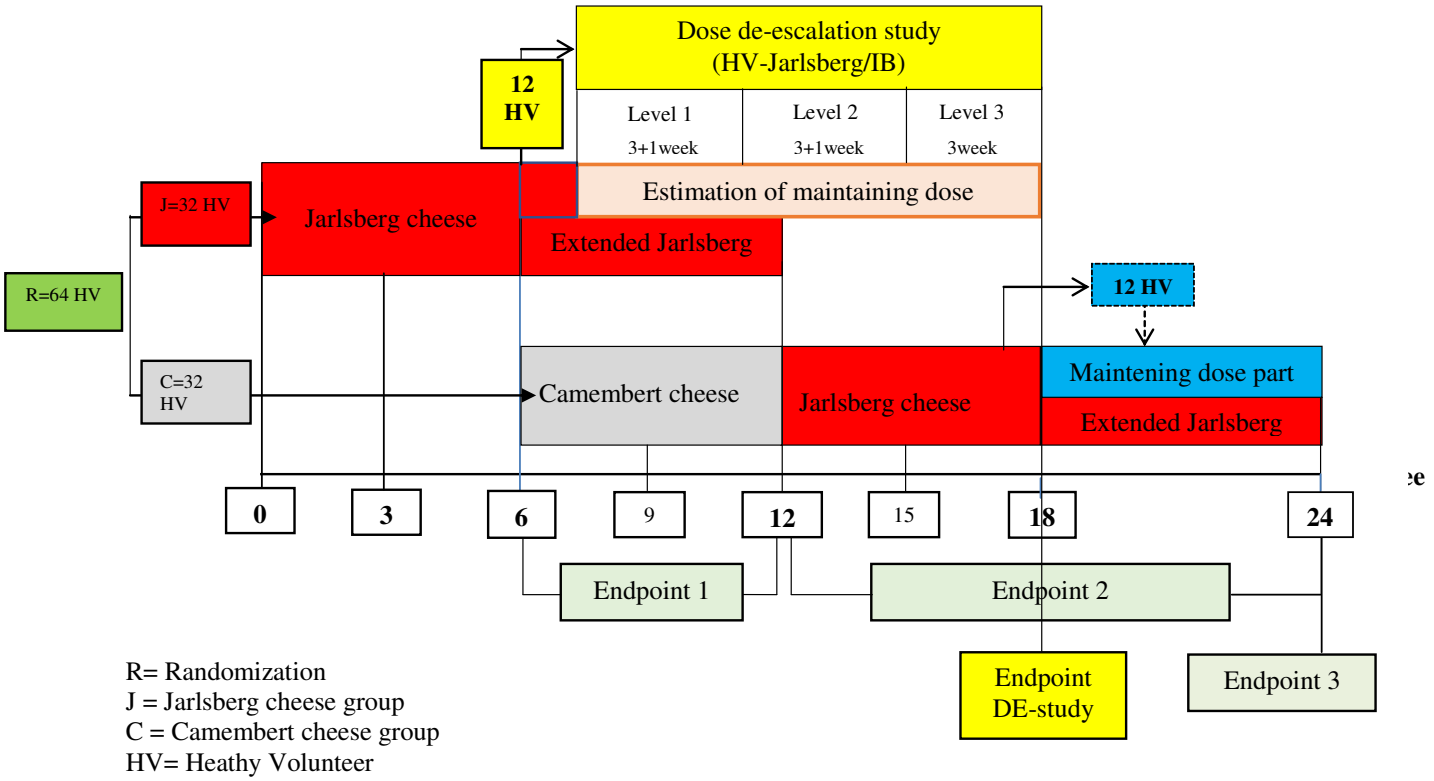

Figure 2: Overall project design; HV-Jarlsberg/III & HV-Jarlsberg/IB

4.2: Comparative study design

The comparative study will be performed with a semi-cross design. The HV will be randomly allocated to either daily intake of Jarlsberg cheese or Camembert cheese without content of vitamin K2. The daily intake of cheese will be 57 g Jarlsberg cheese and 50g Camembert cheese. These doses are nearly equal regarding total energy, protein, carbohydrate and fat. The dose to be used is based on the previous dose-response study of Jarlsberg cheese performed in HV [12].

Research protocol HV-Jarlsberg/III

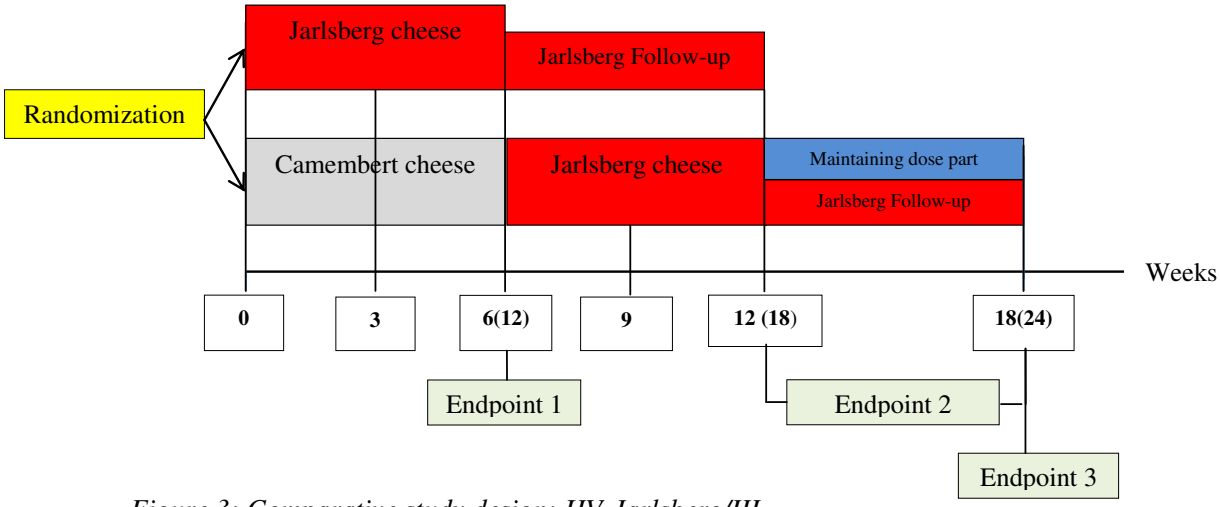

Figure 3: Comparative study design; HV-Jarlsberg/III

The participants meet for clinical investigations at screening Day 0 and after 3 and 6 weeks (Fig 3). At the end of this 6th week, the HV allocated to Camembert cheese will be switched to daily intake of Jarlsberg cheese for additional 6 weeks with clinical investigations every third weeks. The participants allocated to Jarlsberg cheese and had performed the 6 weeks of daily intake of Jarlsberg cheese will be offered participation either in the dose de-escalating study HV-Jarlsberg/IB (separate protocol) or in the extended part with unchanged daily dose of Jarlsberg cheese.

4.3: Extended Jarlsberg period

The participants performed the 6 weeks of daily intake of Jarlsberg cheese either by randomization or switching to Jarlsberg cheese will be offered participation in either:

1) an extended study of 6 weeks with unchanged dose of Jarlsberg
2) a de-escalation dose study of 12 weeks (HV-Jarlsberg/IB protocol)
3) a maintaining dose study of 6 weeks duration to verify the results from the de-escalation study

HVs with an increase <10% in the osteocalcin level from screening to 6 weeks of Jarlsberg cheese intake will not be offered inclusion in the de-escalation study.
The first 12 HVs finalized 6 weeks with daily intake of Jarlsberg cheese and obtained an increase in osteocalcin from baseline ≥10% in the comparative study will be allocated to the de-escalation study HV-Jarlsberg/IB.
The remaining HVs from the comparative parts will be offered participation in the extended part of this study receiving an unchanged daily dose of Jarlsberg cheese (Fig 3). The duration of this part is 6 weeks with clinical investigation at the end of the study.

4.4: Verification of the estimated maintaining dose

The 12 first HVs switched from Camembert to Jarlsberg cheese; finalized 6 weeks with daily intake of 57g Jarlsberg and obtained an Osteocalcin increase ≥10% will be offered participation in the study part aiming to verify the maintaining dose obtained in HV-

Jarlsberg/IB study (Fig 3). The duration of this part will be 6 weeks with clinical investigation at the end of the study.

4.5: Randomization

The volunteers will be randomized 1:1 to either daily intake of Jarlsberg cheese or a Camembert cheese by using block randomization with random block size between 2 and 10 [13].

4.6: Identification of Volunteers

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

Digit 1 & 2: Indicate the GP-site [01=site 1; 02 site= 2 etc.]

Digit 3 & 5: Indicate the number of the patient within each GP-site [001, 0 02 etc.]

V: Evaluation

5.1: Main variables

The main variables in this study are total osteocalcin, carboxylated and under carboxylated osteocalcin and the ratio OR= [carboxylated / under carboxylated] osteocalcin, measured in blood serum. Additionally, vitamin K1 and the different fractions MK 7, 8, 9 and 9/4H will be recorded as part of Vitamin K2.

5.2: Laboratory variables

Blood sample for measurement of the hematological and the clinical biochemical variables will be taken every sixth week. The list of variables to be measured is given below.

5.2.1: Clinical chemistry

The following variables will be measured in serum:

Lactate dehydrogenase (LDH)
Alkaline phosphatase (ALP)
Amylase
Creatinine
Albumin
Urea
Total Bilirubin
ALAT
HbA1c
K^+
Na^+
Ca^{++}
Magnesium
Phosphate
Vitamin D
Total Cholesterol
HDL cholesterol
LDL cholesterol

5.2.2: Hematology

The following hematological variables will be measured:

Hemoglobin (Hgb)
Erythrocytes
Hematocrit
Mean Cell Volume (MCV)
Ferritin
Thrombocytes
Leucocytes
Diff. count:
Neutrophils
Eosinophils
Basophils
Monocytes
Lymphocytes

Blood samples for measurement of osteocalcin and vitamin K will be taken every third week

5.3: Common Terminology Criteria for Adverse Events version 4.0

The CTCAE is divided in 26 System Organ Class (SOC) in accordance with the MedDRA classification [14]. Within each SOC, adverse events (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)

5.3.1: Grading and classification of Items

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 is shown 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 medication are as follows:

Unrelated: bears no relation to timing of medication, similar to symptoms or signs expected in the disease process, does not recur on re-challenge.

Possibly: bears relation to timing of medication, similar to 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: clear relation to timing of medication, distinct from symptoms or signs expected in the disease process, recurs on re-challenge.

5.3.2: Serious Adverse Event (SAE)
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 have to 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

5.4: Factor and vital sign

The participant factors recorded in the study will be age in days from birth to the screening visit calculated in the database, height in cm, and body weight in kg. Additionally, concomitant disease and treatments will be recorded. The 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 minutes rest.

VI: Study procedure

6.1: Trial treatment

In the first part of the study, half of the participants will have a daily intake of either marketed common Jarlsberg cheese with the composition specified in Table I. The second half of the participants will have a daily intake of a cheese with none or strongly reduced contents of vitamin K_2 with the composition specified in section 2.3.

6.1.2 Administration and doses

The daily intake of cheese to be taken is based on the results obtained in the previous dose-response study [12]. The recommended Jarlsberg dose is 57 gram/day representing 5 slices and 50 gram/day of Camembert cheese. The duration of the comparative study part will be six weeks. The duration of the expanded Jarlsberg part or the maintaining dose verification part will be minimum 6 weeks and maximum 12 weeks. In the expanding Jarlsberg part, the participating HVs will use the same dose as in the comparative part. The dose used for the HVs participating in maintaining dose will be determined from the de-escalation study HV-Jarlsberg/IB.

6.1.3 Cheese supply, packing and storage

The Jarlsberg cheese will be delivered in 100-gram packages and the Camembert cheese in 3x50-gram pieces free of costs from Tine. The participants will receive the cheese at the GP-sites at every 3-week or 6-week visits. Both the Jarlsberg- and the Camembert cheese used in the study will be given free of charge to the participating volunteers

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 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 healthy volunteers
EudraCT number	2019-
Protocol id	HV-Jarlsberg/III
Storage	Refrigerator between 4° to10°C

6.2: Inclusion and start-up visit

The recruited HV fulfilling the inclusion and avoided the exclusion criteria will be registered and asked to participate in the study. The participants who are willing to sign the informed consent form will be enrolled in the study. The participants will undergo a clinical investigation and an appointment for the starting visit in the study one week later. All the voluntaries will be instructed not to change their usual intake of food during the study, but change the usual used cheese with the study cheese.
A new clinical investigation with blood sampling will be performed at the start-up denoted as Day 0. During this visit, vital signs, concomitant diseases and treatment will be recorded. Blood samples will be handled in accordance to the GP center procedures, and the Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 registered. In addition to hematological and biochemical variables, the blood samples will be used for measurements of vitamin D, triglycerides, LDL- and HDL cholesterol, osteocalcin, vitamin K1 and vitamin K2.

6.3: Comparative part of the study

The intake of cheese starts the following day named Day 1. New visit will be performed after 3- and 6-weeks intake of the study cheese. During every visit, vital signs will be measured, adverse events (AE) and concomitant medication recorded. Blood sampling for measurement of osteocalcin and vitamin K will be taken every third week, but a complete measurement including both hematology and biochemistry will be performed after every 6 weeks of cheese intake. 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 take action. The intake of cheese will be stopped and the HV will be followed up until disappearance of the symptoms. 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.
The duration of the first part of the study will be 6 weeks for the participants randomized to Jarlsberg cheese and 12 weeks for those allocated to the comparative group. Within one week after the last clinical investigation in this comparative part of the study, the participants will be informed about the obtained results related to change in the Osteocalcin level. The HVs will be offered to continue in one of the following study parts:

1) Expended Jarlsberg cheese part with unchanged cheese dose
2) De-escalation dose study (HV-Jarlsberg/IB; separate protocol)
3) Verification of the estimated maintaining Jarlsberg dose

HVs with an increase ≤ 10% in the Osteocalcin level from screening to 6 weeks of Jarlsberg cheese intake will not be offered included in the de-escalation study. The 12 first HVs completed 6 weeks of Jarlsberg cheese intake obtaining an increase ≥ 10% in the osteocalcin level will be offered participation in the de-escalation study HV-Jarlsberg/IB.

6.4: Expanded Jarlsberg cheese part

The remaining participants fulfilling 6 weeks of Jarlsberg cheese intake will be offered participation in the expanded Jarlsberg cheese part of this study HV-Jarlsberg/III or verification of the maintaining daily Jarlsberg dose. The HVs participating in the expended part will continue the intake of Jarlsberg cheese in additionally 6 weeks with unchanged daily dose. New visit in the study will be performed at the end of the study. During this final visit, vital signs will be measured, CTCAE and concomitant medication recorded and blood sampling performed.

6.5: Verification of the estimated maintaining dose

The HVs switched from Camembert to Jarlsberg cheese; finalized 6 weeks with daily intake of 57g Jarlsberg cheese and obtained an increase ≥ 10% in the Osteocalcin level may be offered participation in study part aiming to verify the maintaining dose obtained in HV-Jarlsberg/IB study. The included HVs will receive the daily maintaining dose recommended from the de-escalation study HV-Jarlsberg/IB for additionally 6 weeks with clinical investigation at the end of the study. During this final visit, vital signs will be measured, CTCAE and concomitant medication recorded, and blood sampling performed.

6.6: Stopping rule

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

6.7: 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 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 in Oslo for analysis.

6.8: 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 has to 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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6.9: Risk and benefit of participation

This study includes only products which is for sale in food stores in the country and proven not to cause any risk of disease except for person with lactose intolerance, allergy against milk product or suffering from a disease for which cheese product has to be avoided. During the comparative part of the study, blood sampling will be performed between three and five times. In the expending Jarlsberg part of the study and the verification of the recommended maintenance dose, blood sampling will be performed two times. Penetration like this will always include a risk of complication. However, all the blood sampling in this study will be performed by certificated and highly qualified bioengineers with long experiences in such work. The risk for failure still exists but classified as very low.
The HV women will receive an economical compensation, free daily use of cheese, coverage of travel expenses related to participation in the study and free medical investigation. Additionally, they will be informed of their osteocalcin level and possible bone density during this study. This might be an important knowledge in case of osteoporosis symptoms later.

6.8: Study duration

The duration of the first comparative part of the study will vary from 6 to 12 weeks depending on allocation to Jarlsberg cheese or the alternative cheese. The duration of the expending Jarlsberg part or the maintaining dose part will be 6 weeks. The total duration of the study will be maximum 18 weeks:

Inclusion of the first participant	13th January	2020
Inclusion of the last participant	24th February	2020
Last participant finalized comparative study part	14th April	2020
Last patient finalized extended part	6th July	2020
Final statistical reports	18th September	2020

VII: Project management and Monitoring

7.1: Project management

The study will be administered by a steering committee consisting of:

- Prof. Stig Larsen; DSc, Clinical Research Methodology and Statistics
  Faculty of Veterinary Medicine, Norwegian University of Life Sciences, Oslo and Meddoc AS, Hvamstubben 14, 2013 Skjetten, Norway

- Dr Helge Lundberg; MD, General Practitioner (GP)
  Skjetten Medical Center, 2013 Skjetten Norway

- Prof. Helge Holo; PhD, Biochemistry
  Faculty of Biotechnology and Food Science, Norwegian University of Life Sciences, Ås Norway

- Hans E Fagertun; MSc Statistician
  Meddoc Research AS, Hvamstubben 14, 2013 Skjetten Norway

- Dr. Trond Holand; PhD fellow
  Faculty of Veterinary Medicine, Norwegian University of Life Sciences, Oslo Norway

Prof. Stig Larsen supported by Dr. Trond Holand will administer the study. Natharat Thiendilokkul (BSc) will perform the clinical monitoring and Vivy L Larsen (MSc) the Data Management. Hans E Fagertun (MSc) and Prof. Larsen will be in charge of the statistical analysis.

7.2: Publication of the results

The results will be published in international medical journals. The steering committee and one investigator from each participating GP-centers will be represented in the list of authors. The rules stated in the Vancouver recommendation will be followed. Prof. Stig Larsen is responsible for the manuscript supported by the committee.

7.3: Quality assurance demands

The study will be performed with electronical data entry by using the InCRF database system. The monitoring part will be daily performed by two monitors at Meddoc and each of the participating GP-centers will be on-site monitored at least two times during the study. Additionally, blood samples for osteocalcin and vitamin K analysis will be collected every third week. The main monitoring will be performed electronically from Meddoc Research and DM will generate queries sending regularly to the sites. The monitor will receive the copy of queries in order to perform the routine monitoring follow up check. Paper CRF's printed eCRF with investigator's signature and copies of the laboratory analysis will be sending by e-

mail to both the DM for performing inhouse data entry and monitor from the GP sites as source documents.
It is the duty of the investigator to provide open access to the monitor to all study related records at previously agreed times and locations.
In conducting the trial, the investigator accepts that the Sponsor, the regional Ethics Committee, the regulatory body and monitor may, at any time, by appointment, conduct an audit of the study site.
In conducting the trial, the Sponsor accepts that the Ethics Committee or the regulatory body may, at any time, by appointment, conduct an audit of the study site, the laboratories conducting any clinical testing or the Good Manufacturing Practice (GMP) manufacturing facilities.

7.4: Investigator meeting

Before application of the trial protocol to the regional ethical committee, a meeting with all the participating investigators will be arranged. The agenda for this meeting will be:

1) Go through the protocol chapter by chapter in order to correct the protocol in accordance with input from the investigators
2) To synchronize the clinical part of the study

Before start of the study and inclusion of the first HV, all the investigators and the responsible persons for data handling at each participating GP-center will be given specified information about the electronic data monitoring system InCRF.

7.5: Electronic data monitoring and training.

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 CRFs will be the source-data.

7.6: Training course

In order to ensure the accuracy of data entry in the InCRF at the sites, one person on each site will be invited to participate in a half-day course organized and performed by the data manager, monitor and the responsible statistician. Additionally, the data manager will visit each GP-center when data-entry of the first HV will be performed.

7.7: Start-up and closing visit

The project manager and the clinical monitor will perform the start-up visit at each of the participating sites.
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.

7.8: 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 eCRFs provided. The CRFs should be made available in the event of a formal investigator site audit.
Each site will be monitored at site two times during the study. Monitoring will be performed mainly electronically. In case of unclear or missing data in InCRF, a list of suggested corrections will be sent to site by Meddoc study monitor.

7.9: Curriculum vitae

The investigators have to submit an updated CV documenting their expertise. The CV has to be signed and dated by the physician and a copy has to be attached to the protocol if required according to international rules. Another copy must be kept in the Trial Master File and a third copy in the Site File.

7.10: Site file

On behalf of the Sponsor, Meddoc AS will supply the investigators with a Site File. The Site File should contain all documents relevant for the study. The investigator is responsible for keeping the Site File updated and secure that all required documents are present in the Site File. The Site File will be inspected during the monitor visits.

VIII: Data Management

8.1. Case Record Forms (CRF)

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 CRF. Source data consist of both printouts from the laboratory examinations and CRF data as baseline characteristics clinical examination collected by the investigator on paper and entered by site. 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.

8.2. 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).

8.3: Data handling

A data entry person at site will enter the CRF data in InCRF and the Data Manager will perform the initial data validation. In case of missing data, logical errors or interpretation problems, a Query will be sent to the site via e-mail for clarification/correction in InCRF. In the meantime, a copy of the query will be sent to monitor for the routine monitoring tasks follow up. When all the needed participants have finalized the clinical part, and the investigator has electronically signed the CRFs, the DM will do the final verification checks and perform final database hard-lock when all errors are corrected.
Screening analysis for logical errors will be evenly performed on this database and errors will be corrected after new information is collected from the site. When all the detected errors are corrected, the main basic database will be locked. The database will be transformed to a labeled SAS database, which also will be locked for 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 has to be re-opened and corrected. The international procedure for such changes will be followed.

IX: Discontinuation

An HV may discontinue the study at any time if, in the view of the investigator, it is in the participant's best interests.
If an HV does not show up to an agreed visit, the investigator should try to motivate her to continue. However, if the HV has decided not to continue, the HV should be asked to attend a control visit as described for the end of the study.

9.1: Discontinuation not related to the study question

A patient who discontinues the study for administrative reasons or reasons documented not related to the cheese intake classifies as "Drop out" and will be replaced by a new volunteer. Drop-outs will be described specially in the statistical analysis and included in the Intention-To-Treat (ITT) analysis by using the procedure "Last observation carried forward" (LOCF).

9.2: Discontinuation related to the study question

HV discontinuing the study for reasons that are related to or might be related to the cheese intake will be classified as "Patient Withdrawal". These participants will not be replaced. They will be included in the Per-Protocol (PP) and the ITT analysis using the LOCF procedure.

X: Ethical consideration

10.1: 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 HV and will only be included in this clinical trial after approval of the trial by the regional Ethical Committee (REK) and after the HVs have received oral and written information and signed informed consent.
The products to be used in this trial are 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 K2 passing a certain daily dose may increase and strengthen the bone tightness and may therefore have a prophylactic effect on osteoporosis. This disease occurs frequently in older population, especially among women passing the menopausal age. Jarlsberg cheese is shown to be one of the Norwegian produced cheeses with the highest level of vitamin K2. The aims of this study are comparing the effect on the Osteocalcin level of Jarlsberg cheese with another cheese without content of vitamin K2. Additionally, to estimate the long-term optimal dose effect of Jarlsberg cheese related to the osteocalcin – and vitamin K2 level and to verify a daily maintenance dose of Jarlsberg cheese in healthy women.
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 seven 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.

10.2: 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.

10.3: 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 volunteers, the trial design and the consequences of participation. Additionally, the

investigators have to obtain written informed consent from the participants before inclusion in the study.

10.4: 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 GP-centers will be anonymous so that the volunteer only can be identified by date of birth, initials and HV's 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 or free. Additionally, they will get the clinical examination for free and receive a moderate economical compensation for the participation. In case they get extra transportation costs for the participation, this will be covered by the study.

XI: Statistical model

11.1: Handling of discontinuation

All volunteers fulfilling the inclusion and exclusion criteria, given informed consent to participate and started with at least one intake of the trial cheese, are classified as included in the study.
In the Intention-to-Treat (ITT) analysis, all these patients will be included and the procedure Last Observation Carried Forward (LOCF) will be used. The participants classified as Drop-out will be excluded from the Per-protocol (PP) analysis.

11.2: Presentation of the result

Assumed continuously distributed variables will be expressed by mean values with 95% confidence intervals [15]. As an index of dispersion Standard Deviation (SD) will be used.
In case of skewed distribution, the variable will be tried logarithmic transformed and the analyses performed on the transformed data. The results will be retransformed for presentation.
Categorized and discrete distributed variables will be expressed in contingency tables [16]. Additionally, important prevalence will be expressed in percentage with 95% confidence intervals constructed by using the theory of Simple Binomial Sequences.

11.4: Comparison of groups

Comparison between and changes within groups will both be performed two-tailed and differences considered significant if the p-values are found less or equal to a significance level of 5%.
For investigation of assumption in distribution of continuous variable, Shapiro – Wilk test will be used [15]. In case of skewness, the variables will be tried logarithmic transformed and the analyses performed on the transformed data.
Comparison of groups with regard to continuously distributed variables will be performed by using Analysis of Variance (ANOVA) weighted for GP-site and the initial observation as covariates [17]. Changes within groups will be performed by using ANOVA model with repeated measurements [18]. Contingency Table Analysis will be used for both comparison between and changes within groups regarding categorized and discrete distributed variables [16].

11.3: Sample size

With a significant level of 5%, a power of 90% and a clinical relevant difference in total Osteocalcin increase of one-time SD between the two groups, at least 48 HV have to be included. By correcting for the number of participating GP and drop-outs during the first part of the study, 32 HV will be included in each group.

XII: Operational matters

12.1: Investigator's agreement

Before start, the primary investigator will confirm the agreements to participate in the trial by signing the Investigator's Agreement Form with the Sponsor.

12.2: Instructions

The project manager, supported by the clinical monitor, will instruct the investigator at the start-up visit and during the study.

12.3: Amendments to the protocol

Changes in the protocol can be required by REK, investigators, project manager or TINE. Changes must be given in written amendments and numbered in 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 participant.

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. They shall check protocol compliance, handling of the test articles and recording of data during the 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

This clinical trial is a precondition for further studies. TINE will therefore use the obtained data and results for marketing the product. The main study database will be stored in the product database of the Sponsor.
The project manager and the investigator may demand and have the right to have the results published in an international medical journal. The draft of the manuscript has to be presented to the Sponsor for comments, discussion and final approval. TINE cannot stop the publication unless it is proved that publication of the results may damage the marketing of the product.

The project manager or the investigator cannot present the results in any meeting or congresses without approval by TINE. The data obtained in this study has to be handled confidentially.

12.8: Investigator and Sponsor withdrawals

The investigator can finalize his or her participation in the study if TINE does not fulfill its duties according to the protocol or the Sponsor-investigator agreement.
TINE has the right to terminate the study at any time. The investigators will be paid according to the agreements in the Sponsor-investigator agreement. A written explanation will be sent to the investigators and REK according to present rules.

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