Nutritional risk assessment for Hip fracture,
A Case control study

Nutrition supplementation of elderly hip fracture patients;
A sub study of an RCT: preoperative and postoperative orthogeriatric service

Thesis by
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2016

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Series of dissertations submitted to the
Faculty of Medicine, University of Oslo

ISBN 978-82-8333-270-4

Cover: Hanne Baadsgaard Utigard
Printed in Norway: 07 Media AS – www.07.no

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Acknowledgements

The work including hip fracture patients in this thesis was carried out at Oslo University Hospital from September 2009 to June 2014. Data from healthy volunteers are also included from 2005-2006 at Oslo University Hospital, Aker. My fellowship was sponsored by Oslo University hospital, Norway.

First off all, I would like to thank the patients, their relatives and the caretakers for participating in this study as well as the staff at the Orthopedic- and acute geriatric ward at Oslo University Hospital, Ullevål. A great acknowledgement goes to all the healthy volunteers participating in the study at Oslo University Hospital, Aker in 2005-2006.

I would especially like to express my gratitude to:

Morten Mowè: My main supervisor, for taking over a seriously sinking project that was stopped by the National Regional Ethical committee. For believing in me and the project and letting me proceed for a second attempt in carrying out this thesis. Morten Mowè, you are the sole medical doctor promoting nutrition in geriatric care and you have been at great importance when discussing the importance of findings and the way forward. I am grateful for your positive outlook on life and for always making our meetings joyful. It has been great working with you, and I hope money will come our way again soon so we can continue our good collaboration.

Torgeir Bruun Wyller: My co-supervisor, I am forever grateful for incorporating my project in your planned hip fracture project. I am thankful for your statistical advice and invaluable help with writing up the papers. For including me in the project group at "Loftet", providing me with a social and scientific network at Ullevål.

Thomas Bøhmer: was the one with the initial idea to first establish this project and took the initiative to this project in 2004. I am thankful that he employed me and gave me great responsibility for carrying out the project. I learnt so much from you Thomas, you gave me a platform to stand on that helped me in carrying out the project at Ullevål.

Knut Strømsøe: was the collaborator with Thomas Bøhmer, being the Ortopedian in charge making the initial project possible recruiting patients and healthy volunteers. I am grateful that you always informed us PhD students in the status of the case against our project and for updating us in the way forward. I am thankful for always listening in to my consearns, your support and belief in me.

Kirsten Solemdal, Gunnhild Bærdal and Gurkirpal Singh for participating in the data collection for the healthy volunteers and for contributing to a joyful working team.

Leiv Otto Watne for being the sole responsible for including patients and collecting data in the Oslo Geriatric Trial that this study springs from. You are wonderfully throw and an example to all of us who wants to do research.

Grete Wang, Clinical dietician, for taking over the nutrition intervention, giving dietary advice to the inpatients while I was in maternity leave, thank you a thousand times!

Research nurses Elisabeth Fragaat, Camilla Marie Andersen og Linda Feldt. I am grateful that you carried out the data collection with such thoroughness and good sense of humor. Missing cases are fabulously low thanks to you!

Anniken Brugaard for her efficient analysis of the medicaments used in the hip fracture patients for the Delirium paper on request from the referee. I am forever grateful.

Anne Garmark for providing all the HR support needed in a speedy response time at all times.

I would also like to thank Thomas Gundersen at AS Vitas, **Kari Julien** at the Hormone Laboratory, and **Anne Hove** at the Nutritional Laboratory, Oslo University Hospital, for their tedious work performing the biochemical analyses for the entire thesis.

And Sophies Minde Ortopedi AS for sponsoring the analysis of the blood for vitamins and bone turnover markers, as well as Takeda AS for sponsoring the supplements.

I also have to thank my family for helping me putting the project in prospective. My son, Thomas 12 year old once said: "Why do you do this mummy? It seems like a lot of work, and not much pay? "I hope this project-time will have taught my kids that life is not all about the money, but what is important is to do something in life that you enjoy doing, that is meaningful to you and to others.

Norsk sammendrag (Summary in Norwegian):

Studien foregikk ved Oslo Universitets sykehus og pasienter ble inkludert fra september 2009 til april 2011. Totalt deltok 116 pasienter og 73 friske kontroller i risikoanalysen for hoftebrudd. Studien er tredelt. I den første delen har vi studert ernæringsmessige risikofaktorer for hoftebrudd i en case control studie. Den andre delen av studien er en del av en større studie der vi har studert effekten av ernæringsintervensjon på vettap, daglig funksjon og benomsetnings-parametere som markører for benhelse i en randomisert kontrollert studie. I den siste delen har vi sett på spesifikke mikronutrienter og deres assosiasjon med risiko for å utvikle akutt forvirring (delirium) under behandlingen av hoftebrudd i en case control studie.

Vi fant at lave mikronæringsstoffer, vitamin A, C, D,E og K1 var assosiert med risiko for hoftebrudd uavhengig av BMI. Lav BMI var også assosiert med hoftebrudd, men vi fant stor spredning i BMI fra 12- 34 i pasientgruppen. Vi fant også en korrelasjon mellom benomsetnings-parametere og spesifikke vitaminer og en synergistisk assosiasjon mellom 25(OH)D og vitamin K1 på risiko for hoftebrudd. Vi konkluderer med at mangel på spesifikke mikronæringsstoffer er assosiert med risiko for hoftebrudd uavhengig av BMI og at noe av risikoen kan forklares gjennom benomsetnings-parametere.

Intervensjonsstudien viser at vitamin K1 og 25(OH)D var signifikant høyere i intervensjonsgruppen sammenliknet med kontrollene, men det var ingen effekt på effektmålene. Intervensjonen hindret et fall i 25(OH)D som kan ha hatt en positiv effekt.

Risiko analysen for å utvikle delirium viste at lave 25(OH) D konsentrasjoner var assosiert med øket risiko for delirium i en multivariat analyse.

Ubehandlet underernæring er vanlig blant eldre i Norge og spesifikk underernæring kan føre til økt risiko for hoftebrudd og bidrar mulig til at Norge ligger på verdenstoppen i hoftebrudd. Omlag 50% av hoftepasienter får et delirium under hoftebruddsbehandling og lave vitamin D bidrar muligens til dette.

Summary in English

The study was conducted at Oslo University Hospital, Norway. Patients were included from September 2009 until April 2011. In total 116 patients and 73 healthy non-fractured controls participated. The study has 3 parts. In the first part, we studied micronutrients and the risk of hip fracture in a case control study. In the second part, we conducted a randomized controlled nutrition intervention trial and finally, in the third part, we studied if micronutrients were associated with delirium in the acute phase of hip fracture in a case control study.

We found that the micronutrients: vitamin A, C, D, E and K1 was associated with increased risk of hip fracture independent of BMI. However, low BMI was associated with hip fracture, but the variation in BMI ranged from 12- 34 in the patient group. Micronutrients correlated with bone turnover markers and there was a synergistic effect of 25(OH)D og vitamin K1 on the risk of hip fracture. We conclude that low micronutrients are associated with increased risk of hip fracture independent of BMI and that some of the association can be explained trough bone turnover markers.

We found that vitamin K1 og 25(OH)D was significantly higher in the intervention group compared with control patients. However, there was no effect on bone turnover parameters between the two groups. The intervention prevented a fall in 25(OH)D that may have had other effects not measured by us.

Low serum 25(OH) D was associated with increased risk of experiencing delirium. Undiagnosed and non treated malnutrition is common in the elderly in norway and spesiffic malnutrition may contribute in the association as to why Norway is world champions in hip fracture. About 50% of hip fracture patients experience a delirium during hospitalization for hip fracture. Low 25(OH)D may contribute to the high risk.

Abbreviations

BALP= Bone Specific Alkaline Phosphatase

BMI= Body Mass Index

BMD= Bone Mineral Density

BTM= Bone turnover markers

CAM= Confusion assessment method

CI= confidence interval

CTX1= C-Telopeptide-Cross-Linked Type I Collagen

HGS= Hand Grip strength

IGF1 = Insulin-like growth factor 1

IL= Interleukin

IQCODE= Informant Questionnaire on Cognitive Decline in the Elderly

IQR= Inter Quartile Range

OOT= Oslo Geriatric Trial

OR= Odds ratio

Pi= Inorganic Phosphate

PINP= Procollagen Type I N-Terminal Propeptide

PPi= Inorganic DiPhosphate

PTH= Parathyroid Hormone

RAR = retinoic acid receptor

RXR = receptor X receptor

ucOC= Undercarboxylert Osteocalsin

VCRE= vitamin C responsive element

VDR= vitamin D receptor

VDRE= vitamin D responsive element

25OHD= 25 Hydroxy Vitamin D

List of papers

This thesis is based on the following original papers:

I: Vitamin K and 25(OH)D are independent and synergistic associated with risk for hip fracture in a Norwegian elderly population, a case control study .

Torbergsen AC, Watne LO, Wyller TB, Frihagen F, Stromsoe K, Bohmer T, Mowe M. Clin Nutr 2015;34:101-6.

II: Micronutrients and the risk of hip fracture: case-control study.

Torbergsen AC, Watne LO, Wyller TB, Frihagen F, Stromsoe K, Bohmer T, Mowe M. Accepted for publication in Clinical Nutrition December 2015. Available on line: http://www.sciencedirect.com/science/article/pii/S0261561415003520

III: Effects of vitamin D, Ca and K1 supplementation upon bone turnover in elderly hip fracture patients. Randomized controlled trial.

Torbergsen AC, Watne LO, Wyller TB, Frihagen F, Mowe,M. Submitted to American Journal of Clinical Nutrition, des 2015

IV: Nutrition related risk factors for Delirium

Torbergsen AC, Watne LO, Wyller TB, Frihagen F, Mowe,M. European Geriatric Medicine . 2015. http://www.europeangeriaticmedicine.com/article/S1878-7649(14)00194-6/pdf.**.**

1. Introduction

The incidence of hip fractures in the Scandinavian countries is high. The numbers reported from Oslo is among the highest in the world (1). Hip fracture is a major health concern for the elderly and the society. 9000 patients are hospitalized and operated due to hip fracture in Norway annually (with 5 million inhabitants) (2).

1.1 Burden and implications for the elderly

The hip fracture patient is often a frail older patient at about 80 years of age and about 75% are female (3;4). Hip fracture often occurs from a low energy trauma resulting from a fall from the patient's own height. This indicates a frail patient often with high comorbidity and long going osteoporosis and sarcopenia. Even if treatment of osteoporosis using bisphosphonates has been associated with increased BMD, osteoporosis most often passes untreated (5-7).

Hip fracture executes a great burden for the elderly fractured patient. About 20 % of hip fracture patients are dead one year after fracture. A reduction in mortality after hip fracture is described only in the oldest age group, aged above85 y. Age, male sex and comorbidity correlate with death, as well as biomarkers for heart, lung and kidney disease (8-10) in hip fracture patients. Increased morbidity after fracture is seen also when adjusting for comorbidity like pressure ulcers, infections, iatrogenic complications (11).

Hip fracture does not only affect physical health, but may also affect mental health. Physical limitations, immobility and pain after hip fracture are easy to predict, less clear is the risk of developing cognitive impairment. Delirium is common in hospitalized elderly patients occurring in 53% of elderly surgical patients and perhaps as many as 80% of elderly surgical patients in the intensive care unit (12). The same figures are reported for hip fracture patients. About 20 - 50% of hip fracture patients experience an acute delirium (13) in the acute phase of fracture. Delirium is related to increased risk of developing cognitive decline (14-16) and the condition frequently passes without detection, an attempt to prevent or treat the condition (12).

It is uncertain if nutrition interventions have any effect in this old and frail patient group. However, using weight as outcome measure, nutrition intervention is likely to be cost effective in hip fracture patients (17).

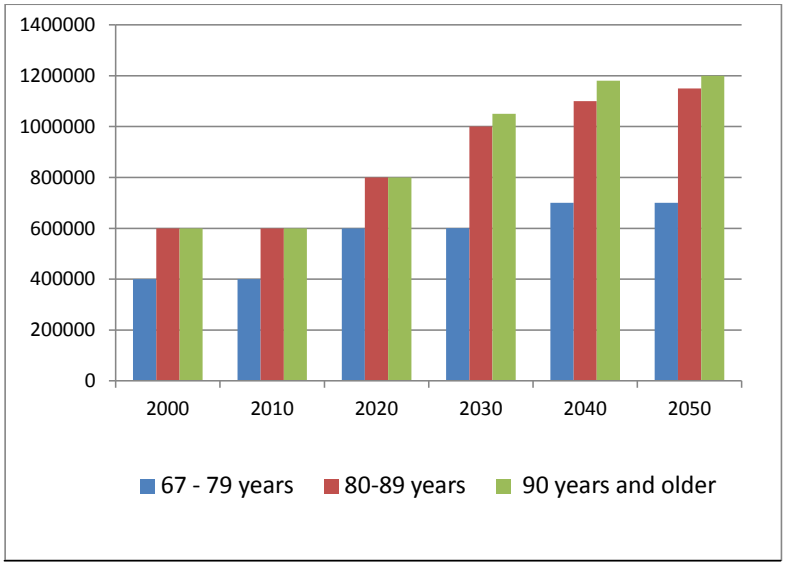
1.2 Burden and implications for the society

The hip fracture incidence increased until 1990. However, Omsland et al reported that the total hip fracture rates declined in both genders during 1999-2008 in Norway, whereas rates of second hip fractures did not change (18).

In Norway today, 6000 hip fractures are of simple nature, treated and valued 250 000 NOK per fracture (23). The remaining 3000 are complicated fractures, valued 675 000 NOK per fracture. The total costs are estimated to 3 billion NOK per year and is expected to increase with 50 – 60 % in 2030 (19). In addition, the total cost is a lot higher when taking into account the cost of reduced daily functioning and need for home health services or the cost of a lifelong institutionalized patient.
The socio economic consequences will be severe in the near future unless good preventative measures are made as well as optimizing the recovery and rehabilitation phase, as sustaining a hip fracture is amongst the greatest risk factor for sustaining a hip fracture (20;21).
Hip fracture occurs later in life due to the slower turnover of cortical bone in the hip compared with trabecular bone in vertebrae and distal forearm (22).

The population growth in the period of 2010-2030 is estimated to 23 % and the Norwegian population will exceed 6 million before 2030. We will see the largest growth among the elderly, an increase of 64 % in the group above 67 is expected and the proportion of elderly above 80 will rice in 2030, see **figure 1** (19). The population growth is expected to continue to rise towards 2050, and is not transient. As a direct consequence, the hip fracture incidence will increase.
About 50% of hip fracture patients experience a delirium (23) during the hospital stay for hip fracture treatment. Earlier, delirium was regarded as a harmless transition condition, but now delirium is associated with the onset of cognitive decline and dementia (14-16;24) as well as condition associated with poor outcome of hip fracture. Due to the increase in population age, more patients will experience hip fracture and delirium.

Figure 1. Expected population growth in Norway



The population of Norway is expected to grow in the forthcoming years. Particularly, the number of elderly in the oldest age groups is expected to grow. The graph is reproduced based on data from the Norwegian Directorate for Health, published in (19).

1.3 Nutrition related risk factors associated with hip fracture

Low Body Mass index (BMI) is a well known risk factor for hip fracture. In fact, the common belief is that it is the very thin and frail looking patients that suffer muscle wasting, osteoporosis and fracture. However it is less well known if the association with low BMI and fracture risk is due to the low padding of these patients and that these therefore sustain hip fracture from low energy trauma, or if it is a lack of specific nutrients that contribute in the association. If it is so that low micronutrient status contributes in increased hip fracture risk, what then with normal and overweight people having an unhealthy diet lacking in fruits and vegetables, fish and lean meat and whole grains and nuts necessary to provide essential micronutrients. Immobilization leads to rapid bone loss, and immobility is common in overweight people.

Vitamin D and K1:

Previously epidemiologic studies have associated low vitamin D and Ca with increased fracture risk (25). The data regarding vitamin D and Ca supplementation upon bone health benefits are convincing and negative results of intervention studies in the past are now being explained by the too low dosage of vitamin D given. The new Nordic recommended intake of vitamin D is 400 IU – 800 IU in elderly / day (10 µg – 20 µg) (25).

Low vitamin K has also been associated with hip fracture over the last decade. The Nurses' health study in 1999 was one of the first studies to identify a link between high dietary vitamin K and decreased hip fracture risk (26). The role of vitamin K in mineralization of bone has become an area of interest over the last decade (27;28).

Few studies have examined the two nutrients, vitamin D and vitamin K combined, although it has been done in Japan (29;30). Both studies report low 25(OH)D and low vitamin K in hip fracture patients and they postulate that the association with risk of hip fracture is independent of general malnutrition. The evidence on vitamin K and fracture risk from Japanese studies are becoming increasingly convincing. However, the possible relation between vitamin K combined with low vitamin D and fracture risk has been poorly explored in European populations. However, a recent RCT in Greeks supplemented with a dairy product fortified with vitamin D alone or vitamin D and physiological doses of vitamin K combined. The largest effect on undercarboxylated osteocalsin and BMD was for vitamin D and K combined (31).

Vitamin C and E:

Low vitamin C was associated with increased risk of hip fracture for the first time in 1995 in Sweden (32). Follow up studies have been performed, however, the data are limited and the literature has not managed to convince initiation of large scale interventions to test the association.

The postulated association between vitamin C and E and hip fracture risk is primarily based on epidemiological data studying fruit consumption, in vitro cell culture studies and animal models. Even so, the Agency for Healthcare Research and Quality's comparative effectiveness Review of Treatments to Prevent Fractures in Men and Women with low Bone Density or Osteoporosis states that the combination of calcium and vitamin C may reduce non vertebral fracture risks in certain populations (33). Also a protective effect upon hip fracture risk by supplemental vitamin C has been reported (34).

Multiple protective mechanisms of action of vitamin C and E are suggested, such as their ability to reduce oxidative stress and inhibit bone resorption. Moreover, vitamin C is necessary for the synthesis of collagen, a protein important for bone quality and tensile strength (34). However, the evidence is insufficient to incorporate vitamin C or vitamin E in regular advice for patients with osteoporosis. A high intake of fruits is reported to be associated with lower fracture risk (35) but it is difficult to know whether this is a direct action of vitamin C or of other components in fruits and vegetables like potassium or bicarbonate or a more indirect correlation as people who eat more fruits and vegetables also tend to have a better lifestyle, i.e. exercising more and smoking less. Lower serum concentrations of vitamin C have been reported in a low income area compared with a high income area of Oslo (36), and low education and socioeconomic status are frequently reported risk factors for osteoporotic fractures (37). This might in part be explained by low intake of vitamin C through fruits and vegetables in low income areas.

The B vitamins:

Deficiencies in vitamin B, along with the consequent elevated homocysteine level, are associated with bone loss, decreased bone strength, high bone turnover and increased risk of hip fracture (38-41). The metabolism of homocysteine depends on several B vitamins including: folate, B12, B2, and B6 (42). The authors of the Hordaland health study indicate that intake of folate, but not vitamin B12 were an independent risk factor for hip fracture (43) It has been reported lower intake of vitamins B1 and B6 in a hip fracture group compared to control subjects, and reduced risk of hip fracture after supplementation with folate and Mecobalamin (44). However studies are few and a prospective study of elderly women with high homocysteine found no increased risk of hip fracture (45). The latest review on vitamin B, Homocysteine and bone health is inconclusive (46).

High Homocysteine is suggested to interfere with the covalent bonds in the triple helix of Collagen affecting Collagen quality. It is suggested that low vitamin B lead to increased Homocysteine that does not only affect bone density but that the interference with the cross-linking of collagen is related to increased risk of hip fracture (42).

However most studies have investigated high Homocysteine, not the vitamin B- family and it is uncertain whether it is the low vitamin B or increased Homocysteine that contributes in the association.

Vitamin A:

Epidemiologic studies have found excessive vitamin A intake and high serum retinol to be a possible risk factor for hip fracture (47-49). Melhus et al described a harmful intake at only twice, 1.5 mg retinol, the daily recommended intake (47). However, some epidemiologic studies have failed to confirm the association (50;51). Holvik et al found no evidence of an adverse effect of high serum retinol on hip fracture. If anything, there was a trend towards an increased risk of hip fracture at low retinol concentrations (52).

Vitamin A refers to any compound exerting biological activity of retinol. Intake of vitamin A through diet is either performed vitamin A in eggs, liver, dairy and supplements, or provitamin A (carotenids) in vegetables converted to performed vitamin A in the body. If vitamin A is not needed in the body, excess vitamin A is stored in the liver and released into the blood when needed.

Numerous cellular functions are mediated through vitamin A, including bone cell functions. Bone mass is dependent on bone turnover and the ability to mineralize newly formed bone. It is well recognized that vitamin A may stimulate bone resorption, but later studies indicate that vitamin A may also inhibit bone resorption and perhaps even stimulate bone formation (53). Few studies have elucidated this aspect, but it has been suggested that vitamin A may act in synergy with vitamin D in bone mineralization (53).

Cod liver oil was previously a good source of vitamin A. The possible association between high vitamin A intake and hip fracture found in 1998 (47) led to a reduction of vitamin A in cod liver oil from 500µl to 250 µl/5 ml. However, recently, the association between hip fracture risk and vitamin A has been found to be U- shaped. Both low vitamin A and high vitamin A has been found to be associated with increased fracture risk (53).

Few supplements contain just vitamin A alone, without vitamin D. If vitamin A is high due to supplementation, 25(OH)D will also normally be high.

Malnutrition is a frequently reported risk factor for hip fracture and no previous studies, to our knowledge, have measured the actual serum retinol in hip fracture patients.

Good sources of vitamins in the Norwegian diet

In the Norkost study, conducted by the Directory of Health, the mean intake of vitamins and minerals were mostly within the Norwegian nutrition recommendations, except for vitamin D and folate which were below the recommended level in both genders. When dietary supplements were taken into consideration, the mean intake of vitamin D and folate was closer to the recommendations. 30 to 40 percent of the participants had a total intake of fruits and berries above the recommended level of at least 250 grams per day. However, the recommended level of vegetables of at least 250 grams per day was achieved by about only 15 %. Also, the intake of food and micronutrients decreases with age. The oldest age group examined was 60 to 70 years, younger than our population (54). **Table 1** reports good sources of vitamins in the Norwegian diet 2010 – 2011.

Table 1. **Good sources of vitamins in the Norwegian diet 2010 – 2011**

Food sources are given in descending order, the largest contributor first.

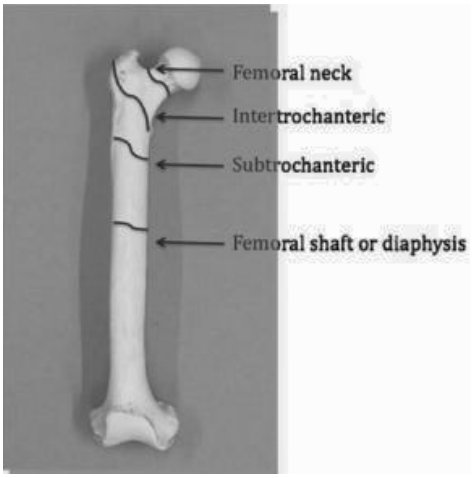
Micronutrient	**Food source**
Vitamin A	Vegetables
	Meat and products of meat
	Cheese
	Butter
Vitamin D	Fatty fish
	Butter / margarine/ oil / fortified milk
Vitamin E	Butter / margarine / oil
	Egg
	Fish and products of fish
Vitamin B1	Bread
	Meat products of meat
	Milk and yoghurt
Vitamin B6	Meat products of meat
	Fruits / Berries
	Bread
	Egg
	Vegetables
Folic Acid	Vegetables and fruits
	Bread
	juice
Vitamin B12	Fish and products of fish
	Meat and products of meat
	Milk and yoghurt
Vitamin C	Vegetables
	Fruit and berries
	juice
Vitamin K1[a)]	Vegetables
	Fruits and berries

a) No data was given for vitamin K1 in the Nordkost study. We used calculated data by Drevon et al based on the Norkost study in 1997 (55).

1.4 Definition of hip fracture

Hip fracture was defined as femoral neck fracture, a trochanteric or a subtrochanteric femoral fracture, **figure 2**.

Figure 2 Hip fracture: specific sites



We included Femoral neck, intertrochanteric and sub-trochanteric fractures. Femoral shaft or diaphysis fractures were excluded as these are usually a result of higher energy trauma (56).

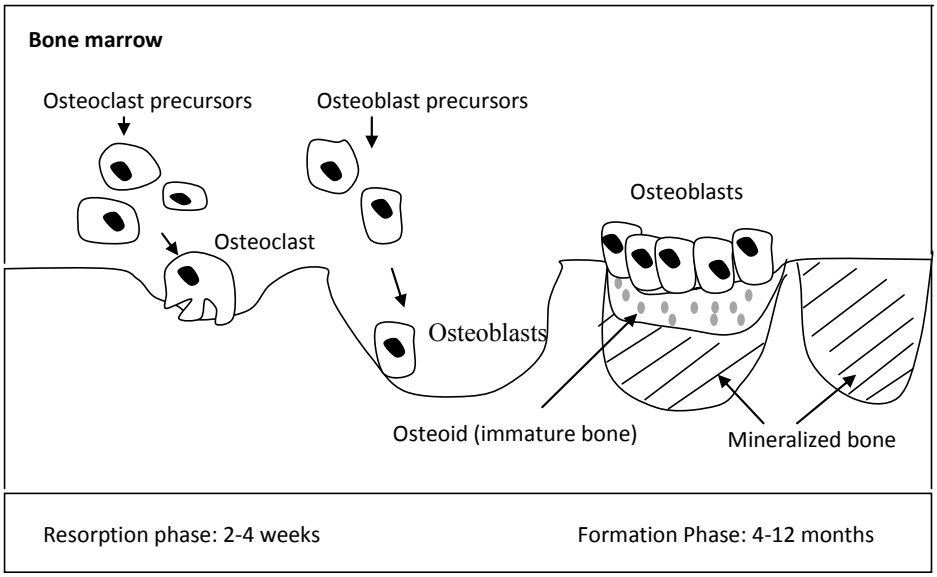
Photo is from the world wide web.

1.5 Biochemistry of bone metabolism, bone turnover and nutritional aspects

The organic matrix of bone consists of 90 % Collagen type I, providing tensile strength in bone, combined with a lesser amount non-collagenous protein, primarily Osteocalsin, a Ca binding protein. The organic matrix is mineralized by the deposition of inorganic Ca and phosphate, and small amounts of various ions in small crystals called hydroxyapatite(22) .

Two types of cells perform bone growth and remodeling: Osteoclasts resorbs bone (bone resorption) and makes the foundation for Osteoblasts that form new bone (bone formation), Bone resorption and bone formation are linked processes. When a fracture needs to be repaired, Osteoclast precursors proliferate into mature Osteoclasts and initiate bone resorption that takes 2 – 4 weeks. Thereafter undifferentiated mesenchymal cells proliferate into mature Osteoblasts. The Osteoblasts first work on matrix maturation, then mineralization. The process can take 4 – 12 months, **figure 3.**

Figure 3: Bone remodeling / bone turnover.



Osteoclasts provide a platform for subsequent bone formation performed by Osteoblasts. In healthy individuals, the process is in balance (22).

In healthy individuals, bone formation and resorbtion remains in balance through a coupled process. At menopause, or age above 40 years, the loss of estrogens triggers excess bone resorption that exceeds the capacity of bone formation and uncouples the two processes. This triggers age related bone loss leading to Osteoporosis, the most common bone metabolic disorder in developed countries (22). The same age related bone loss is seen in men, however at a slower paste. Adequate nutrition and exercise inhibits the process and maintain bone mass.

Osteoporosis is characterized by:

- Loss of bone mass
- Microarchitectural deterioration of bone tissue
- Increased fracture risk

Bone turnover

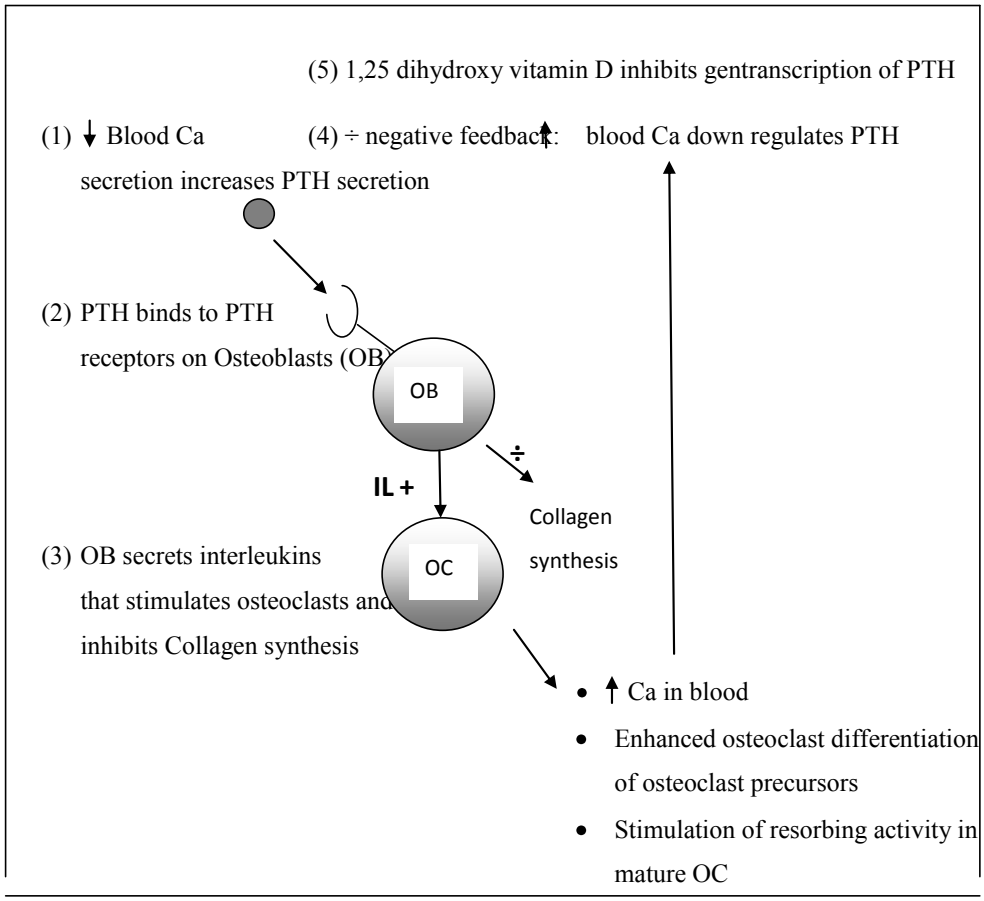
Parathyroidea hormone (PTH) and 1.25 dihydroxy vitamin D are regarded as the primary hormones regulating bone mass, securing homeostatically controlled Ca in blood by acting on bone, kidney and the intestine:

PTH

PTH is synthesized and secreted by the parathyroid glands. Low extracellular free Ca stimulates PTH synthesis and secretion. PTH receptors are found on Osteoblasts, not

Osteoclasts and the bone resorption stimulating effect of PTH is indirect through interleukine stimulation of osteoblasts resulting in increased extracellular Ca that exerts a negative feedback on PTH secretion and metabolism. The stimulatory effect on osteoblasts by PTH also down regulate the synthesis of Collagen from Osteoblasts. 1,25 dihydroxy vitamin D decrease PTH secretion by suppressing PTH gentranscription, see **figure 4.** Prolonged PTH eventually also stimulates osteoblast activity.

Figure 4: Effect of PTH and 1,25 dihydroxy vitamin D on bone:



PTH = Parathyroid Hormone, OB= osteoblasts, OC= osteoclasts, IL= Interleukines

The figure is drawn from text provided in (22).

Vitamin D

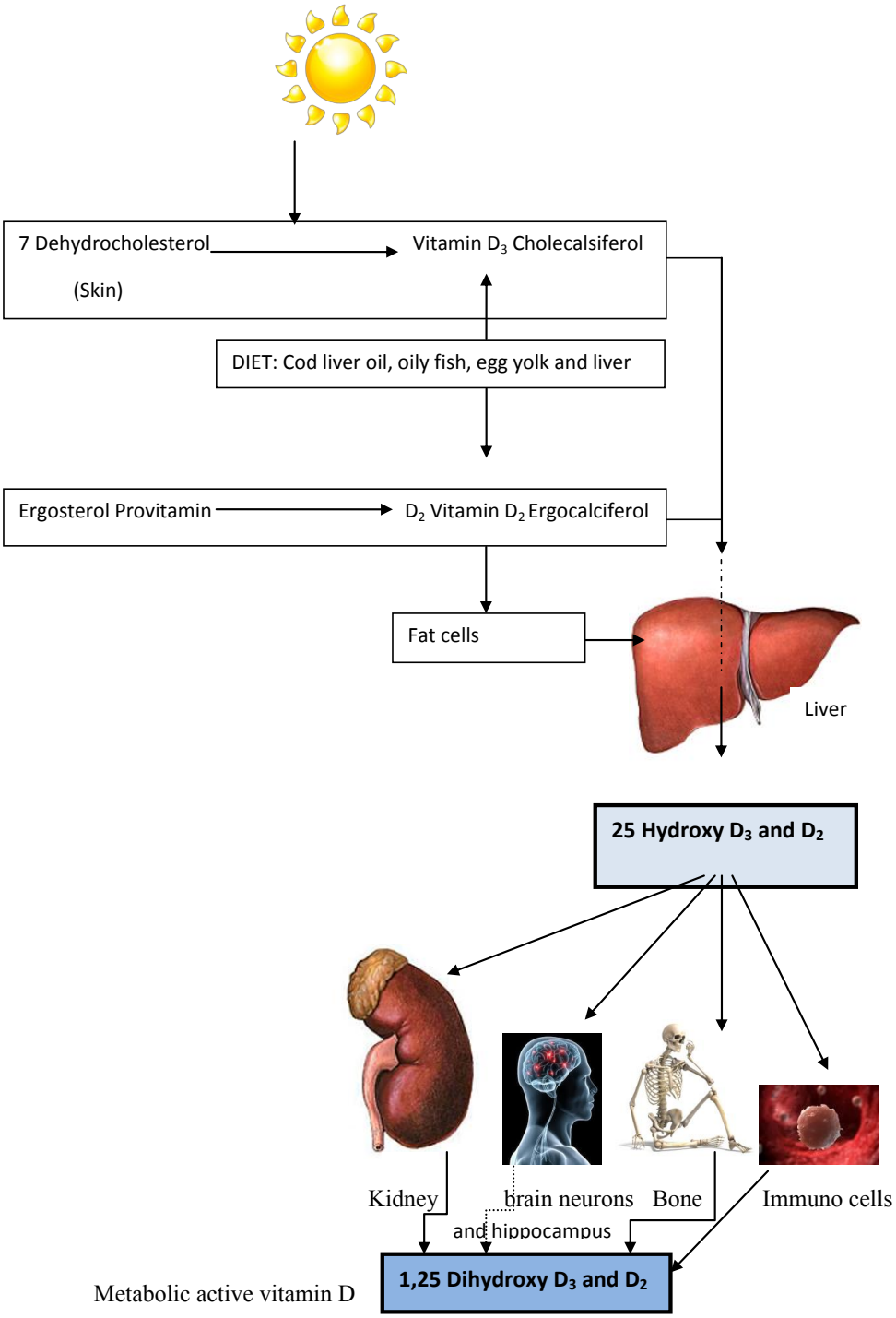
The term vitamin D refers to both vitamin D_2 and vitamin D_3. Good sources of vitamin D are through sun exposure, dietary intake of fish oils and oily fish, egg yolk, liver and dietary supplements. In Norway the only food supplemented with vitamin D is semi skimmed milk (0.4 µg per 100 g). Vitamin D both through sun exposure and dietary intake needs activation by the liver to 25 hydroxy vitamin D (25(OH)D) and hydroxylases to 1,25hydroxy vitamin D 1,25(OH)D. Previously it was thought that the final activation of vitamin D happened solemnly in the kidney. However recent data suggests that other tissues like bone cells and immune cells expresses vitamin D hydroxylase and expresses autocrine/paracrine effects on both bone and inflammation, see **figure 5** (57). The active form of vitamin D is 1,25 dihydroxy vitamin D with a half life of 4 – 6 hours, less than 1 % of 25(OH)D and circulates at 1000 folds lower concentration of 25(OH)D. 1,25(OH)D is under homeostatic control. As 25(OH)D concentration declines, secondary hyperparathyroidism increases and the circulating 1,25(OH)D maintained until severe substrate depletion arises. Consequently, serum levels of 1,25(OH)D are not reduced in many patients with vitamin D deficiency. Therefore, 25(OH)D, but not 1,25(OH)D reflects fracture risk and is suitable for measuring vitamin D status (58-60).

The main objective for vitamin D is to maintain Ca homeostasis. At low Ca concentrations PTH stimulates the formation of the 1,25 form of vitamin D leading to increase absorption of Ca from the intestine High 1,25 vitamin D exerts a negative feedback on PTH synthesis.

1,25 vitamin D also exerts anabolic effects on bone by stimulating osteoblast activity to secrete bone specific alkaline phosphatase (BALP) and osteocalsin that mineralizes newly formed osteoid.

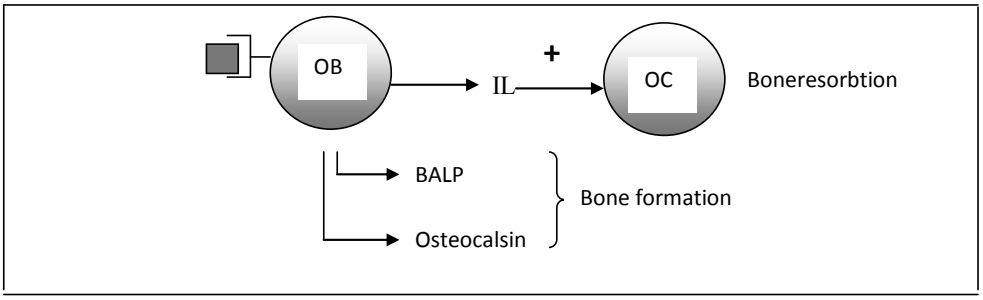
It is considered safe to supplement using 800 IU or 20mg vitamin D in the elderly, and it is indeed recommended (25;57). High doses of vitamin D may be potentially toxic (61). Adverse effects of ultra high doses >50 000 IU cholecalsiferol daily is found in several reports with serum 25(OH)D at 370 nmol/L (62;63). A randomized controlled trial allocating elderly to eighter 500 000 IU cholecalsiferol annually or placebo resulted in increased number of falls, especially in the first 3 months after supplementation, and higher rate of fracture (64). One reason may be due to that vitamin D supplementation may increase muscle strength and mobility, but not balance at least initially. (65).

Figure 5. Sources and activation of vitamin D (66) The illustration is assembled using pictures from the world wide web.



Older persons have a decreased production of cholecalciferol in the skin and a decreased absorption of vitamin D in the gastrointestinal tract (67).
Vitamin D stimulates bone formation but also bone resorption indirectly through stimulation of IL secretion by the Osteoblasts, see **figure 6**.

Figure 6: 1.25 vitamin D and action on Osteoblasts



1,25 vitamin D binds to vitamin D receptors on Osteoblasts.

■ = 1,25 vitamin D, BALP= Bone Specific Alkaline Phosphatase , OB = Osteoblasts, OC= Osteoclasts

Most cells responds to vitamin D through a nuclear vitamin D receptor (VDR) found in most body tissues (66). VDR are essentially nuclear transcription factores, able to bind directly to DNA turning specific gens on or off. The VDR receptor commonly makes heterodimers with the Retinoic X receptor (RXR) that also forms dimmers with the retinoic acid receptor (RAR). This suggests that vitamin A may be important in bone metabolism together with vitamin D (22;53).

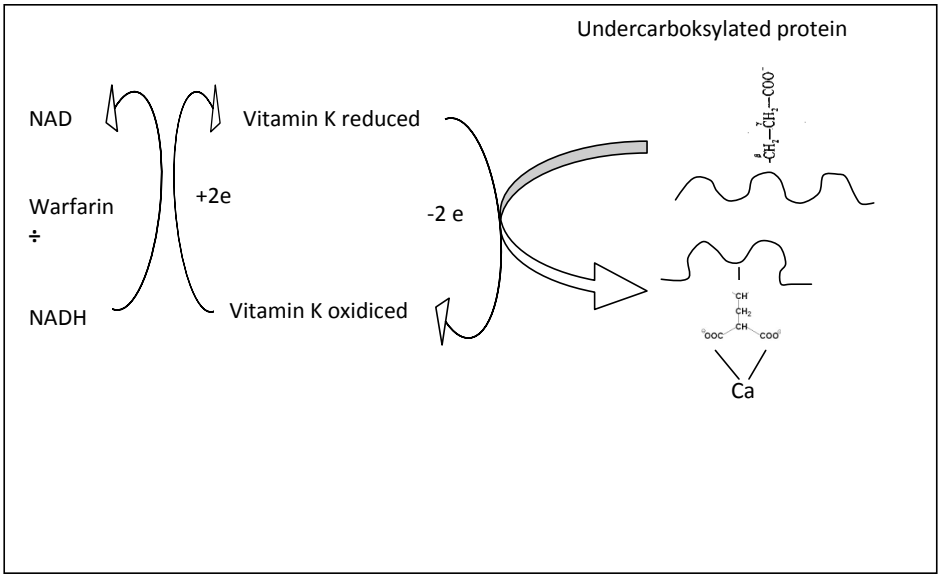
Reference range for vitamin D: 25(OH)D > 50 nmol/L give no rice in PTH. When 25 vitamin D drops below this level, PTH increases and bone resorption occurs (22). The new Nordic recommendations for 25(OH)D concluded based on a literature review that 25(OH)D > 50 nmol/L is sufficient vitamin D status (25). This is confirmed by others (68).

Vitamin K

There are two groups of vitamin K, vitamin K1 phyllokinone mainly found in green leafy vegetables and vitamin K2, a serious of compounds either produced in the intestinal colon or consumed from soya fermented foods, cheese and meet. The vitamin Ks are known to help regulate calcium content in several tissues, see figure 7 (66). Vitamin K donates electrons to gamma glutamyl carboxylase to enable the enzyme to carboxylate selected amino acids in the protein. The vitamin K becomes oxidized and is reduced in an enzyme facilitated process using electrons from nicotinamide adenine dinucleotide (NADH). Warfarin blocks this reducing step of vitamin K and effectively inhibits carboxylation of all vitamin K dependent

proteins, including proteins important for bone mineralization, as the body has limited body stores of vitamin K. Gamma carboxylation of vitamin K dependent proteins results in three-dimentional relocation of the protein, activating the protein usually to able it to bind Ca.

Figure 7: Oxydation/reduction of vitamin K drives protein carboxylation



E= electrons, NADH = Nicotine Adenine Dinucleotide Reduced, NAD= Nicotine Adenine Dinucleotide Oxidiced. Warfarin inhibits the recycling of oxidized vitamin K back to reduced active vitamin K

Bone turnover markers (formation and resorption)

Bone turnover markers (BTM) increases in the transition state pre to postmenopausal women. Thereafter BTM return to premenopausal levels but increases with age (69). All BTM in an elevated state, measured in this thesis are associated with osteoporosis (70). Ivasaka et al found no difference in BTM measured shortly after fracture (within 2 days) compared with pre fracture data (71). This indicates that BTM measured within hours of hip fracture, like we did, reflects pre fracture levels. However BTM are increased 24 weeks post fracture (72). Seasonal variation in BTM is plausible due to the dependence on vitamin D that varies throughout the year. However Michelsen et al found no such variation in a large scale cross sectional study aiming to establish reference intervals for BTM (69). A nocturnal variation in BTM Bone has been reported, however this was not found in postmenopausal women (69).

Formation:

Markers of bone formation are products of osteoblast activity, the protein synthesis of Collagen and Osteocalcin:

- PINP or PICP: The amino or carboxyl terminal of propeptide of type I Collagen
- BALP: Bone specific alkaline phosphatase
- Osteocalsin

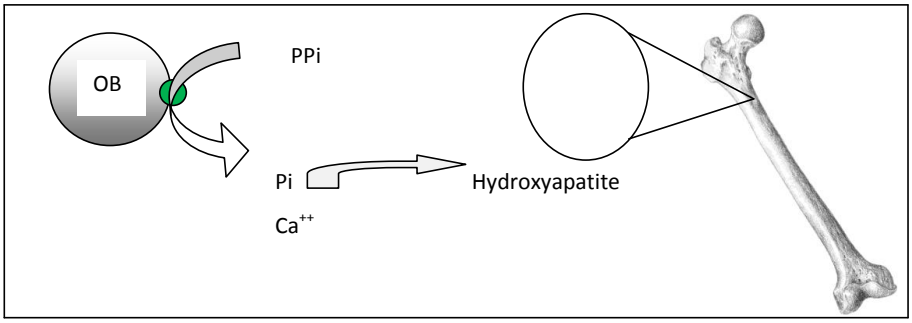
Procollagen Type I N-Terminal Propeptide (PINP) and Procollagen Type I C-Terminal Propeptide (PICP):

The Collagen contains a triple helix bound by covalent bonds at specific amino acids. At each end of the molecule, N and C terminal, there are no bonds and no helix. These endings, the propeptides PINP and PICP, are removed postranlatoric and released into the blood as an indirect measure of collagen synthesis. PINP is released before mineralization and increases more compared with PICP during active bone growth.

Bone specific alkaline phosphatase (BALP):

BALP is a membrane bound enzyme on Osteoblasts that cleaves inorganic diphosphate to inorganic phosphate. BALP is produced during the matrix maturation phase when newly formed collagenous matrix is prepared for mineralization. In addition to Ca, inorganic Phosphouros are incorporated in bone mineralization and forms hydroxyapatite crystals, **figure 8**. The balance between PPi and Pi is thought to be crucial for bone mineralization as PPi inhibits the formation of hydroxyapatite. The release of BALP into blood is delayed and it takes time before plasma BALP increases and reflects bone formation. BALP is increased in metabolic bone disease, including osteoporosis. BALP has a long half life of 40 hours with no diurnal peak.

Figure 8. Bone specific alkaline phosphatase (BALP) activity

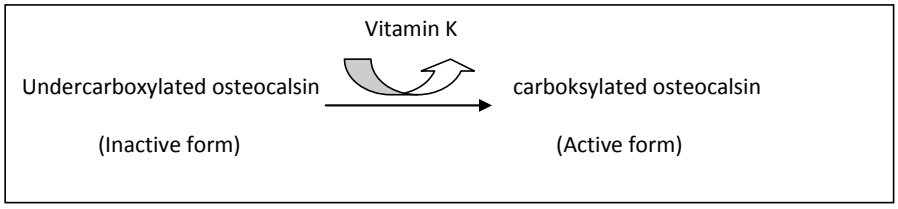


● = BALP = Bone specific alkaline phosphatase, OB= Osteoblast, Pi=inorganic Phosphate, PPI= inorganic diphosphate.

Osteocalsin:

Osteocalsin is also known as bone Gla protein, the most abundant non collagenous protein in bone.
Osteocalsin undergoes a vitamin K dependet postranslatoric process where the protein is being carboxylated into the active form that binds Ca in hydroxyapatite**, figure 9**, and may be important in bone mineralization. Osteoclasts have a half life of 5 minutes and have a nocturnal peak.

Figure 9. Vitamin K activation of osteocalsin



The recommended daily intake of vitamin K in the USA is 90-120 µg /day. This level is based on that this intake is enough to maintain coagulation (66). However, Booth et al found no effect on bone health after supplementation with 400 IU vitamin D in combination with 500 µg vitamin K1 and 600 mg Ca for 3 years. The authors suggest that the dosage vitamin K1 may have been too small as 10 – 40 % of osteocalsin remained undercarboxylated at this intake. A larger dosage may be required to obtain adequatesy in bone health (73).

Resorption:

Markers of bone resorbtion are breakdown products of type I Colagen. They includes the amino (N) terminal and carboxy (C) terminal cross linked telopeptide parts of collagen:

- NTX (N- telopeptide)
- CTX 1(C- telopeptide)
- 1CTP (C- telopeptide of type I collagen)

We limit the overview to C-Telopeptide-Cross-Linked Type I Collagen (CTX1), measured in our papers.

Cross linked telopeptides are released into the blood during bone resorption because cross links resists proteolysis. Proteolysis in the C terminal produces CTX that reflects bone resorbtion. Telopeptides are increased in osteoporosis.
CTX1 has a diurnal peak and is affected by food consumption. Sampling times should be standardized.

The International Osteoporotic Foundation recommends one bone formation marker, PINP and one bone resorption marker, CTX1 to be used as reference markers of bone turnover.

PINP and CTX1 are recommended as the most informative bone turnover marker for monitoring osteoporosis in the current Euraopean guidance for the diagnosis and management of osteoporosis in postmenopausal women (74).

1.6 Vitamin D and muscle strength

Vitamin D prevents falls by supporting skeletal muscle health. Vitamin D deficiency induces skeletal muscle weakness (myophaty) that causes gait instability. Muscle tissues carries VDR and the myophaty may be reversal with vitamin D supplementation as double blinded RCTs supplementing with vitamin D increases muscle strength and balance reducing the risk of falling (75).

1.7 Delirium

The term delirium was first used by Celsus in a medical setting in the first century bc. Now, 2000 years later the pathophysiology of this medical condition is still a mystery (76).

Delirium, also known as an acute confusion state, is characterized by altered mental status. The changes appear rapidly and the symptoms vary. Any patient can experience delirium, however, the frailer the patient, the smaller trauma is needed to initiate the condition. The patient may express little or no symptoms at daytime, but might be severely confused and uneasy at night. The condition may last from a few hours to several months, but per definition it should be a passing state.

The core symptoms in delirium are inattention and cognitive impairment. Changes in consciousness, disoriented thinking, disturbances in perception, delusions, hallucinations and changes in sleep-wake cycles are frequently noted (77)

There are two groups of delirious symptoms (78):

1) Hyperactive delirium characterized by agitation, hyperactivity, aggressiveness, hallucinations and delusions.
2) Hypoactive delirium characterized by reduced responsiveness, retarded speech and psychomotor speed and little facial expressions,

Clinicians often think of delirium as a hyper active state. Therefore patients who experience a hypo active delirium often pass unnoted. As the incidence of delirium increases with age, old patients with acute illness should be assessed for delirium daily using the Confusion Assessment Method (CAM) (79), (Textbox 1).

Delirium is associated with the onset or worsening of cognitive decline, being institutionalized for the first time and increased mortality (80-83). It has been shown that delirium is a preventable condition and possible to treat and it is reasonable to believe that preventing delirium may also reduce mortality and other conditions related to delirium (84-86). Although the more predisposing factors you have like: cognitive decline, chronic illness, taking a high number of medications and having sensory impairment the lesser the trauma is needed to trigger a delirium.Inn tidligere

Delirium in hip fracture patients

Hip fracture patients constitute a frail patient group of old age with high comorbidity and hip fracture is regarded a major trauma despite that the majority of hip fracture patients included in studies is low energy fractures. About 20 -50 % of hip fracture patients experience a delirium (13;23) during treatment for hip fracture. A 5 year follow up study of hip fracture patients found that 72 % of patients who experienced a delirium during hospitalization for hip fracture were dead compared to 35% mortality amongst patients who did not experience a delirium during hip fracture treatment (87). There is evidence that delirium is harmful in itself, but delirium is also an important marker for underlying frailty.

Nutrition related risk factors for delirium

Hip fracture patients are frequently reported to be at risk for malnutrition and malnutrition has been associated with delirium (85;88). We previously reported that low BMI was associated with an increased risk for delirium in hip fracture patients (13). However, it is poorly examined if it is the low BMI per se or if the association is due to specific micronutrients. Daily consumption of antioxidants, the B vitamins as well as fish and omega-3 rich oils has been associated with a decrease in dementia (67;89), however it is uncertain if the micronutrients are associated with delirium.

Low vitamin D has achieved attention as a potential contributor in the pathogenesis of delirium as vitamin D receptors has been located in the human cortex and hippocampus, which are key areas for cognition and may regulate immuno modulation including anti-inflammatory and antioxidant effects (90-92). These studies did not find that low vitamin D was associated with increased risk of delirium (although at borderline significance). The leading pathophysiological theory of delirium is related to inflammation and that delirium is a

consequence of reduced anti – inflammatory activity (93) and a low 25(OH)D has recently been associated with increased inflammation (94).

This theory indicates that the antioxidants vitamin C, E and A may also contribute in the mechanism of developing delirium, but no such studies has, to our knowledge, been carried out to test this hypothesis.

The B vitamins has been associated with cognitive decline, but few studies have examined the vitamin B status and delirium. In a case report, persisting delirious state resolved after B12 injection (95) and a study supplementing mild cognitive impaired patients with vitamin B12 found a reduction in delirium (96). However, the association is poorly examined and needs further elucidation.

1.8 New organizational models for treatment for hip fracture

New organizational models for treatment for hip fracture are being developed due to the frail nature of hip fracture patients (97). These patients are at multiple risk of comorbidity leading to an advanced medical need and follow up after fracture in addition to fracture repair (11). The new models seem promising but needs to be tested further using sound scientific methodology (23;98). The hip fracture patients included in this nutrition related risk analysis, is a sub study of a randomized controlled trial of the patient related outcomes of an orthogeriatric service designed to treat hip fracture patients in the preoperative as well as the postoperative phase (97).

A study from Sweden found reduced incidence of delirium after treatment using protein enriched meals as part of a multi professional intervention and low protein energy intake has been reported during the first postoperative days after hip fracture operation (99). Protein enriched meals may be warranted also in treatment of hip fracture patients (100;101). The vitamin and mineral supplementation for bone health was based on an attempt to achieve adequate vitamin D, K1 and Ca as these were the micronutrients with the strongest reported association with hip fracture reported in the literature (38).

2. Aims of the study

The overall aim of this thesis was to gain more knowledge about nutritional risk factors in aged hip fracture patients. In adittion we had three specific aims:

1) Study nutritional risk factors for hip fracture. We wanted to examine if low vitamins: A, B, C, D, E and K1 was associated with hip fracture risk and if found associations could be confirmed through bone turnover markers. Paper: I and II

2) Evaluate if nutrition supplementation for 4 months in the RCT could induce difference in weight loss, difference in daily functioning in the intervention group compared with controls and detect differences in BTM between the two groups. Paper: IV.

Furthermore we wanted to 3) Study if micronutrients were associated with risk of delirium in hip fracture patients. Paper: III

3. Patients and methods

For patient flow chart, see appendix 2

3.1 Inclusion criteria:

All patients admitted to Oslo University Hospital, Ullevål with a low energy trauma resulting in a fracture to the hip were assessed for eligibility. Low energy trauma was defined as fall from own height or from a level not higher than 1 meter. One recent fracture in addition to the hip fracture (e.g. radius or shoulder) was accepted. The catchment area for the hospital was the city of Oslo, Norway. The patients were randomized to the acute geriatric ward (intervention) or to the orthopedic ward with care as usual (controls) in the emergency room prior to operation by the orthopeadic surgeon on call.

3.2 Exclusion criteria for the RCT:

- Hip fracture as part of a multi trauma
- Patients near death at operation (judged by the admitting orthopaedic surgeon)
- Missing consent or assent

3.3 Patient population and blood sampling for paper I,II and III

Eligible patients were patients included in the Oslo Orthogeriatric Trial (OOT). The patients were enrolled in the period from September 2009 to April 2011. 116 patients were enrolled for preoperative blood analysis at baseline. The exact number of each vitamin and BTM analysis varied due to that not enough blood was collected to complete all blood analysis. Due to the large volume required for analysis of vitamin C (2.5ml serum eqvivalent of 1 blood glass), vitamin C was the first vitamin to be down prioritized when there was a shortage of blood.

The patients in the risk assessment for delirium was the same as for the risk assessment for hip fracture, n= 116 patients.

3.4 Control group for paper I and II

73 control subjects, individuals with no previous fracture of the hip (66 % female), were recruited from the census files of Oslo in 2005. Control patients were predominantly home dwelling inhabitants aged 60 - 100 years (median age 82 years). The control subjects were approached by letter, followed up by two phone calls.

3.5 Patient population and blood sampling for paper IV

Patients were reassessed in their homes by a blinded to treatment group project nurse at 4 months follow up of the fracture. All 216 patients included initially were eligible patients for 4 months follow up, regardless of their participation at baseline. 71 patients (31 intervention patients and 40 controls) were included for the primary analysis of the nutrition intervention.

65 patients without baseline values had blood drawn at four months, contributing to supplementary unadjusted analyses thus comprising 136 patients (66 intervention patients and 70 controls).

3.6 Blood sampling

At baseline, blood was collected by venipuncture shortly after admission for the hip fracture and prior to operation. All samples clotted 30 min at room temperature and serum was separated by centrifugation. Aliquots were immediately stored at -80°C, and later analyzed for assays of vitamins and bone turnover parameters.

In the control group of healthy elderly, blood was collected in the morning by venipuncture following an overnight fast. Processing of the blood was as for patients.

A designated nurse, blinded to randomization, visited all patients in their homes at 4 months follow up in order to collect blood for analysis. Blood was drawn and aliquots were blocked for sunlight and stored at -80°C within 1 hour for assays of vitamins and bone turnover parameters.

3.7 Data collection:

The data were collected by designated project staff in both patients and in non fractured controls. In patients, weight was measured using a class 3 chair scale, patients wearing light clothing on the first possible day after operation. Height was either measured using a tape measure towards a wall or calculated from measured knee height (KH) (102), flexing the knee so that the angle between foot and leg was 90 degrees and measuring from the anterior surface of the thigh near the patella to under the heel. As weight was measured shortly after operation, it was anticipated to reflect also the body weight before the fracture. Civil status was categorized in widowed or non-widowed, (never married or divorced were grouped together with non-widowed as these subjects probably have a social network that is independent on spouse); residence in home dwelling or institutionalized; education level in elementary school or more than elementary school; smoking habits in current smokers or not current smokers; and alcohol consumption in total abstainers and non abstainers. The number of drugs used was recorded and activities of daily living was measured using the Barthel ADL Index (BADL) (103). Hand Grip Strength (HGS) was examined by hand dynamometry (Jamar, Germany, three repetitions per examination) in the dominant arm. Patients were examined daily throughout the duration of the hospital stay. The best handgrip test was used for analysis. Delirium was assessed daily throughout the hospital stay using the Confusion Assessment Method criteria (79), textbox 1. In patients without delirium, assessment was stopped after 5 days.

Data collection in healthy controls was performed as for patients and civil data categorized as for patients, but standing height was measured with a tape measure towards a wall and weight

was measured using a class 4 standing scale patients wearing light clothing. In controls, the best HGS result of three repetitions was used. Delirium was not assessed in control subjects.

At for months follow up, a blinded to treatment allocation nurse visited the patients in their home. Weight was measured using a portable standing scale by, patients wearing light clothing. The same portable scale was used in groups, interventions and controls. Height was measured towards a wall using a tape measure. The chair scale and the portable scale were calibrated before, during and at the end of 4 months follow up. Barthel ADL Index (BADL) (103) was used to measure daily functioning and handgrip strength (HGS) was examined by hand dynamometry (Jamar, Germany, three repetitions per examination) in the dominant arm. The best handgrip test was used as for baseline.

3.8 The nutrition intervention

Intervention pre operatively:

The nutrition intervention was a part of a multiprofessional intervention in the acute geriatric ward.

Prolonged preoperative fasting was actively prevented. We offered two nutrition protein enriched drinks (2x200ml) daily and self-selected food for the meals during the waiting time for operation until 6 hours before operation. Water, lemonade and carbohydrate enriched drinks was offered until two hours before surgery.

Intervention post operatively:

We offered protein energy enriched meals (35 kcal/kg body weight) throughout the hospital stay after operation. Food of choice was offered and reduced sized portions to patients with poor appetite. We offered two nutritional and protein drinks daily (2x 200 ml), calorie and protein content depended on the patients preference for nutrition drink, and supplemented with Nycoplus Ca, K1 and D two tablets daily, each containing:

- 75 µg vitamin K1,
- 5 µg vitamin D_3
- 500 mg calcium
- (only vitamin D and calcium for patient using oral anticoagulants)

In addition, we supplemented with 5 ml cod liver oil or two cod liver oil capsules/ or gel pads daily containing:

- 0.7 g Omega – 3 – fatty acids
- 10 Vitamin D_3
- 250 µg vitamin A
- 10 mg vitamin E

Control group:
The control patients randomized to the traditional care in the orthopedic care received traditional treatment at the orthopedic ward. The nutritionist never entered the orthopedic ward in order not to contaminate the nutrition intervention. Nor did control patients receive any supplementation. Due to ethical reasons, they were not asked not to take supplements or increase protein and energy intake.

However the control patients were admitted to the same rehabilitation institutions as the intervention patients.

3.9 Analysis of vitamins in serum / whole blood

Standard preoperative blood analyses were examined in all patients, n= 335, according to surgical protocol, including: CRP, Albumin and Hgb. These were analyzed by a multi analyzer Cobas Integra 800 from 2009 to Oct. 2011, thereafter by Cobas 800 (Both from Roche diagnostics, Mannheim, Germany) in the Department of Clinical Chemistry at Oslo university Hospital, Ullevål.

In controls, the same parameters were assayed with a Modular P 800 multianalyzer (Roch diagnostics, Mannheim, Germany) in the Department of Clinical Chemistry at Oslo University Hospital, Aker.

The vitamins and BTM were analyzed using the same laboratories and using the same assays in patients and in controls:

Vitamin K1: Vitamin K1 was analyzed in serum by **Vitas AS Norway** (www.vitas.no) using high pressure liquid chromatography (HPLC) with on-line electrochemical reduction and fluorescent detection.

25(OH)D: was analyzed by the **endocrine laboratory at Oslo University, Aker.** We measured 25 hydroxy vitamin D, figure 6, as 25(OH)D is less affected by day to day variations due to the long half life of 2-3 weeks and 25(OH)D and is regarded a good measure for body vitamin D (22). 25(OH)D was analyzed by the endocrine laboratory at Oslo University, Aker, using radioimmunoassay (RIA), (DiaSorin, Stillwater, MN USA).

The following vitamins were analyzed at the Nutrition laboratory at Oslo University Hospital, Aker Norway:
The analysis of vitamin C was performed within 14 days after sampling; the others were analyzed continuously within 2 months after sampling.
Vitamin A: Laboratory assays of vitamin A (retinol) were performed using Bio-Rad Laboratories kit, (Munich, Germany).
Vitamin C: The analysis of vitamin C was performed according to Zannoni et al (104).
Vitamin E: Vitamin E was analyzed by Radio Immuno Assay (RIA) from Bio-Rad Laboratories (Munich, Germany).
Vitamin B1 (Thiamin): Vitamin B1 was analyzed in full blood using different assays for patients and healthy non fractured controls:

- Patients: High pressure liquid chromatography (HPLC) was used (Chromsystems, Munich, Germany)
- Healthy non fractured controls: High pressure liquid chromatography (HPLC)was used according to Tallaksen et al (105).

(Thiamin could therefore not be included in the risk assessment for hip fracture, but was included in the 4 months follow up study and in the risk assessment for delirium).

Vitamin B6 (Pyridoxal-5'-phosphate) In patients at baseline, vitamin B6 was analyzed both in serum and in fullblood both by High pressure liquid chromatography (HPLC),(

Chromsystems, Munich, Germany). At 4 months follow up, vitamin B6 was assayed in fullblood only by HPLC (Chromsystems, Munich, Germany).

In healthy non fractured controls, vitamin B6 was analyzed in serum only HPLC, (Chromsystems, Munich, Germany).

(In the nutrition risk analysis serum comparisons were made. In the 4 months intervention study, and in the delirium study, fullblood comparisons were made).

The following vitamins were analyzed at the central laboratory at Oslo University Hospital, Aker Norway:

Folic acid and vitamin B12 (Cobalamin):

Folic acid and vitamin B12 were assayed with a Hitachi 717 Modular multianalyzer from Boehringer Mannheim, (Mannheim, Germany), performed in the Department of Clinical Chemistry at Oslo University Hospital, Aker, Norway in both hip fracture patients and in healthy non fractured controls.

3.10 Analysis of bone turnover markers in serum

Undercarboxylated osteocalsin (ucOC): was analyzed by enzyme-linked immunosorbent assay (ELISA) kit cat#MK118 (Takara Bio Inc. (Japan) by Vitas AS Norway (www.vitas.no).

The following was analyzed by the endocrine laboratory at Oslo University, Aker Norway

Total osteocalsin (totOC): was analyzed using non- competitive immunoluminomeric assay (LIMA), Immulite 2500 from Siemens Healthcare Diagnostics, (Los Angeles, CA USA).

Bone specific Alcaline Phosphatase (BALP): was measured by enzyme activity immune extraction, kit from Metra Biosystems Inc., (Ca USA). BALP was quantified in E/L, 1E= 1 μmol p-NPP hydrolyced / minute, where p-NPP is a monoclonal anit-bone-ALP antigen.

Parathyroid Hormone (PTH): was analyzed by immunoluminomeric assay (LIMA), Immulite 2500 from Siemens Healthcare Diagnostics, (Los Angeles, CA USA).

Insulin-like growth factor 1 (IGF1): We used enzyme-linked immunosorbent assay (ELISA) to analyze IGF1 using kit from Medix Biochemica, Finland.

The following was measured in patients only and was therefore only included in the intervention study:

C-Telopeptide-Cross-Linked Type I Collagen (CTX1): was analyzed by enzyme-linked immunosorbent assay (ELISA), kit from IDS, Denmark

Procollagen Type I N-Terminal Propeptide (PINP): was analyzed by Radioimmuno assay (RIA) kit from Orion Diagnostics Oy, Epsoo, Finland.

Reference values:

The normal reference value assigned by the laboratory for the vitamins and BTM are reported in table 2 and 3 respectivly. The inter coefficient of variance for the vitamins and BTM are reported in table 4 and 5. The inter coefficient of variance have remained stable over time, the methods did not change unless stated, and we used the same laboratories during the entire project period.

Table 2. The normal reference value assigned by the laboratory for the vitamins and bone turnover markers

Nutrient	**Reference area**
Vitamin K1	0.10-2.20 ng/mL
25(OH)D in adults	37-131 nmol/L
Vitamin A	1.2 – 3.6
Vitamin C	45 – 100 µmol
Vitamin E	17.0 – 45.0 µmol/ L
B1 old method	55.0-125 nmol/L
B1 new method	109- 195 nmol/L
B6 fullblood	40 – 160 nmol/L
B6 serum	> 15 - 160 nmol/L
B12	140 – 600 nmol/L
Folic acid	> 8 nmol /L
UcOC	Not established
TotOC	< 3.6 nmol/L
BALP	15-43 E/L
PTH	1.4-8.6 pmol/L
IGF1	7.0 – 29 nmol/L
CTX1 in women in men	 < 1.35 µg/L < 0.75 µg/L
PINP in women in men	 15-96 µg/L 22-87 µg/L

ucOC= undercarboxylated osteocalsin, totOC= total osteocalsin, BALP= Bone specific Alcaline Phosphatase, PTH= Parathyroid Hormone, IGF1= Insulin-like growth factor 1 CTX1= C-Telopeptide-Cross-Linked Type I Collagen and PINP= Procollagen Type I N-Terminal Propeptide.

Table 3: Inter coefficient of variance for the vitamins and bone turnover markers:

Nutrient	Inter coefficient of variance
Vitamin K1	13-20%
25(OH)D in adults	16
Vitamin A	4.5
Vitamin C	2.6
Vitamin E	3.1
B1 old method	6.2
B1 new method	4.2
B6 fullblood	2.5 - 3.2
B6 serum	2.5 – 3.2%
B12	6.8
Folic acid	9.7
ucOC	13-20
totOC	7
BALP	2
PTH	8
IGF1	12
CTX1	10
PINP	11

ucOC= undercarboxylated osteocalsin, totOC= total osteocalsin, BALP= Bone specific Alcaline Phosphatase, PTH= Parathyroid Hormone, IGF1= Insulin-like growth factor 1 CTX1= C-Telopeptide-Cross-Linked Type I Collagen and PINP= Procollagen Type I N-Terminal Propeptide.

3.11 Power calculations and statistics:

Power calculations: Differences in vitamins between cases and controls were based on: $N= 2(Ó/\Delta)^2 x\ f(\alpha\beta)$, Ó= predicted stdv for observations, Δ is clinical expected difference, α= 0.05 type one error and β is 80 % power (106) for the nutrition risk assessment and primary aim.

A reference sample for vitamin K1 and 25(OH)D in healthy home dwelling citizens of Oslo was established in 2005. Estimated means ± SD for vitamin K1 and 25(OH)D in hip fracture patients were obtained from a pilot study. Power calculations were based on estimated difference in means between the established reference population and the pilot study: 0.31 ng/ml and 17 nmol/L for vitamin K1 and D, respectively and SD of the means. We needed a sample size of n= 26 and n= 50 subjects, for vitamin K1 and 25(OH)D respectively to detect differences of this magnitude at 80% power with a 5% significance level for the two vitamins. The reference sample and the pilot study also included data for vitamin C and E. Vitamin D was the limiting vitamin.

To account for uncertainties in the estimated mean differences in vitamin concentrations in the pilot study and the need for 10 extra cases per confounder in multivariate analyses, we decided to increase the number of subjects.

Even so, the study was not powered to run all vitamins in the same analysis simultaneously and adjusting for all statistically significant confounders for hip fracture.

The rule of thumb for extra 10 cases in a regression analysis does not apply when multiple variables correlate and express a common condition. In our case the vitamin deficiency as do low BMI represents malnutrition. Spearmans Rho between vitamins did not achieve 0.7, but most of the vitamins correlated at about 0.4-0.5. We could therefore not run all vitamins in the same logistic regression analysis simultaneously.

Statistical analyses were performed in SPSS 18 (SPSS Inc, Chicago, IL) for Windows.

Comparing groups: In all papers, Student's *t* test or Mann-Whitney *U*- test was used to compare continuous data between groups, and Chi-squared test to compare categorical data. All *P* values are 2-tailed.

Correlation analysis: Spearman's rho or Pearson correlation coefficients were calculated to explore correlations between continuous variables.

Logistic regression Due to statistical power considerations and high correlations between vitamin concentrations we initially examined 25(OH)D and vitamin K1 separately as 25(OH)D is the micronutrient best established with risk of hip fracture and the increasing evidence that the effect of 25(OH)D seem to be linked with vitamin K.

Crude and adjusted ORs for hip fracture were estimated by logistic regression analyses. A number of possible confounding factors detected in the univariate analysis were adjusted for in the multivariate analysis. In order to study interaction between vitamins, the data was grouped in high versus low vitamins K1 and 25(OH)D. The Cut off for vitamins was set to the shortest CI for each vitamin.

In the multivitamin assessment for risk of hip fracture, we built two binary logistic regression models. In model I, all statistically significant explanatory factors other than the vitamins of interest for this paper were included and then removed in a backward manner. In this model we also included vitamin K1 and 25(OH)D as we previously reported these to be a risk factor for hip fracture (107). In model II, all vitamins studied in this paper (vitamin A, C, E, B) were included. Finally, we built three logistic regression models, one for each of the vitamins that had demonstrated an independent and statistically significant association with fracture risk in model II.

Vitamins examined both preoperatively and at 4 months follow up were: vitamin K1, 25(OH)D, vitamin B1, B6, C, E and A. BTM examined both preoperatively and postoperativly were: The bone formation markers: totOC, ucOC, the BALP, IGF1 and PINP. For bone resorption markers, we used CTX1 and PTH. In the primary analysis a change in vitamins and BTM was assessed using paired comparison and the difference between intervention and the control group was adjusted for baseline data. A supplementary analysis was provided comprising all patients measured at 4 months follow up but without baseline data in an attempt to rule out selection bias and to increase statistical power.

In the risk assessment for delirium continuous explanatory variables were initially categorized in quintiles as recommended by Hosmer (108), and the linearity of the relationship between the outcome and the dependent variable was examined. When clear threshold effects were displayed, with a decrease in the odds for delirium when the explanatory variable exceeded a certain level, the relevant explanatory variable was categorized accordingly. Other explanatory variables were treated as continuous. 25(OH)D was hence dichotomized at 50 nmol/L, a cut off regarded sufficient in the New Nordic Recommendations for the elderly

(25). In a multivariate logistic regression model was carried out in order to identify independent risk factors.

3.12 Ethical considerations

Patients, or the nearest next of kin, gave informed written consent before enrollment; some patients were included based on presumed consent, confirmed by nearest next of kin shortly after enrolment. Patients with delirium upon admission, that got better throughout the hospital stay, were presented with a simplified written information letter and were given the ability to give or withdraw the presumed consent. Patients lacking a valid informed consent or assent were excluded. The participants in the healthy control group all gave written informed consent.
Taken that the hip fracture patients today do not get nutrition supplements, we considered it to be no harm leaving half of the patients in the traditional orthopedic care receiving no nutrition supplement.

The study was performed according to the Helsinki declaration. The Regional Committee for Medical Research Ethics, the Data Inspectorate and the Directorate for Health and Social Affairs approved the study protocol.

4. Main results

4.1 Micronutrients and the risk of hip fracture (paper I and II)

During the inclusion period, 116 patients out of 216 eligible patients were included in the study. Missing patients were due to that there was not enough time for blood sampling for project purposes prior to operation, low capacity to draw blood at weekends, holidays and at night. A logistic regression analysis was run for missing. No confounders were significantly associated with missing. This analysis suggests that missing patients are completely at random. Low BMI was, as expected, associated with increased fracture risk, but the vitamins did not correlate with BMI and the patient group was a frailer group compared with controls. Frailty and BMI variables were thus adjusted for in the multivariate analysis and could not explain our findings:

Paper I: Vitamin K1 and 25(OH)D are independently and synergistically associated with risk for hip fracture in an elderly population, a case control study (107).

The vitamins K1 and 25(OH)D were associated with increased risk of hip fracture. The adjusted odds ratios (95% CI) per ng/ml increase in vitamin K1 was 0.07 (0.02 – 0.32), and per nmol/L increase in 25(OH)D 0.96 (0.95 – 0.98). TotOC were lower and BALP were higher in patients compared with controls. Both vitamin K1 and 25(OH)D correlated positively with totOC and negatively BALP. However, even though statistically significant, the correlations were low and cannot explain in total the BTM in patients. The odds for hip fracture with low vitamin K1 increased 3 times in the subgroup of low 25(OH)D compared with high 25(OH)D

Conclusion: Low serum levels of vitamin K1 and 25(OH)D are independently associated with an increased risk of hip fracture. There is a significant synergistic effect between the two nutrients, possibly mediated through OC. Vitamin K1 and 25(OH)D may be important in preventing development of osteoporosis and in treatment of hip fracture patients to reduce the risk of subsequent fracture, especially in patients with pronounced co-morbidity

Paper II: Micronutrients and the risk of hip fracture: case-control study (109)

The vitamins A, C and E were independently associated with a risk of hip fracture in a multivariate regression model. The adjusted odds ratios (95 % Confidence interval) per 10 µmol/L increase for vitamin A was: 0.75 (0.8–0.9); per µmol/L vitamin C: 0.94 (0.91 – 0.98) and vitamin E: 0.87 (0.80 – 0.96). A log transformed vitamin B6 remained associated with increased risk of hip fracture when all statistical confounders were adjusted for, but not when adjusting for vitamin C or vitamin A. The vitamins A, B6, C and E correlated positively with totOC and negatively with BALP. Vitamins B12 and folic acid were not associated with risk of hip fracture nor correlated with BTM. Out of all the vitamins (including vitamin K1 and 25(OH)D, vitamin C expressed the strongest correlation with totOC, Spearmanns rho = 0.41 at $p < 0.001$. This indicates that vitamin C may be more important for the production of osteocalsin than previously assumed.

Subanalysis for home-dwelling patients and institutionalized patients was performed. Vitamin C and vitamin B6 were significantly lower in institutionalized patients compared with home-

dwelling patients (p=0.05 and p=0.02) respectively. However, the OR for hip fracture remained for vitamins A, C and E when the multivariate model was run for home dwelling patients only.

Conclusion: Low vitamin A, C, and E concentrations were associated with an increased risk of hip fracture, also when 25(OH)D, vitamin K1 and BMI were adjusted for. The association is possibly mediated through bone turnover mechanisms. Supplementation with vitamins K and D may not be sufficient in order to optimize bone health and prevent hip fracture.

4.2 Nutrition intervention (paper III)

Effects of vitamin D and K1 supplementation upon bone turnover in elderly hip fracture patients. Randomized controlled trial Submitted to American Journal of Clinical Nutrition, des 2015

Paper III: The Oslo Ortogeriatric trial was negative with respect to prevention of delirium (23). Therefore, the intervention and control groups were merged for the purpose of the delirium study.

At 4 months follow up, the patients in both groups (the intervention group at the acute geriatric ward and the control patients treated in the orthopedic usual care unit) lost weight and scores in BADL. No difference were seen between the groups. Vitamin K1 and 25(OH)D were significantly higher in the intervention group compared with the controls: Vitamin K1: 1.0 ±1.2 vs 0.6 ±0.6 ng/ml, p=0.09, 25(OH)D: 60 ±29 vs 43 ±22 nmol/L, p= 0.01 when adjusted for baseline differences. However, there was no difference in BTM between intervention and controls. In the intervention group there was a non-signifficant increase in 25(OH)D, whereas in the controls there was a significant decrease in 25(OH)D from baseline to 4 months follow up. The BTM correlated with vitamin K1, 25(OH)D, vitamin C and E, confirming an association between vitamins and BTM reported in paper I and II.

Conclusion: The supplementation of 25(OH)D and vitamin K1 improved the serum concentration of these vitamins, but this did not translate into any improvement in the bone turnover markers.

4.3 Micronutrients and the risk of delirium (Paper IV)

Vitamin Deficiency as a Risk Factor for Delirium (110).

Paper IV: 24 patients withdrew from the study before the four moths follow up. In addition, from 18 we did not obtain blood samples at 4 months because the patient did not cooperate in blood sampling or because an insufficient amount of blood was drawn as to complete the nutrition sub analysis and 3 patients were excluded from the analysis as these had only serum vitamin B6 analyzed at baseline, thus leaving us with 71 patients (31 intervention patients and 40 controls) for the primary analysis of the nutrition intervention. There was no significant difference in number of missing patients between the intervention and the control group. Out of the 24 missing from the OOT: In the control group, 8 were dead, 1 was too ill to participate, 4 were not willing to participate and 1 was missing due to other reasons. In the control group 5 were dead, 1 was too ill to participate, 3 were not willing to participate, 1was missing due to other reasons. 65 patients without baseline values had blood drawn at four months, contributing to supplementary unadjusted analyses thus comprising 136 patients (66 intervention patients and 70 controls). Vitamins were lower in the 24 patients that dropped out compared with patients with complete data.

Out of all the patients included in the nutrition sub analysis, 49% experienced a delirium throughout the hospital stay. Out of the vitamins examined, only the marker for vitamin D, 25(OH)D and vitamin C were significantly lower in the delirium group compared with the non delirious group: nmol/L 25(OH)D: 45.9 ± 22.1 vs 55.1 ± 26.7, p = 0.05 and μmol/L vitamin C: 25.1 ± 16.4 vs 35.2 ± 19.5, p= 0.01 respectively. Dichotomizing 25(OH)D at cut off 50 nmol/L, low 25(OH)D was associated with increased risk for delirium: OR= 2.7 (95% CI 1.0 – 6.9), p= 0.04 in a multivariate regression analysis adjusted for all registered confounders. The association for vitamin C remained significant when all known confounders but pre fracture dementia was adjusted for.

Conclusion: Insufficient serum concentrations of 25(OH)D may be causally linked to the development of delirium in hip fracture patients.

5. Discussion

5.1 Nutritional risk for hip fracture Paper I and II

It has long been known that low vitamin D and Ca are associated with hip fracture risk and the association is as good as confirmed through PTH and bone resorption (111-113). A large meta analysis concludes that vitamin D and Ca reduces risk of fracture by 18% (114). The numbers of hip fracture reported from Oslo is among the highest in the world (1;115). A study in Sweden found that hip fracture incidence increased by 3.0% (95% CI: 2.7-3.2%) per degree increase in latitude for men and by 1.9% (95% CI: 1.8-2.1%) for women. There was a marked seasonal variation of hip fracture with the highest risk in February and lower during the summer. The associations found with latitude and season is consistent with a role of vitamin D in hip fracture causation (116). This is contradicting the common belief that the incidence of hip fracture is not affected by seasonal variation and slippery sidewalks in winter in Norway due to the fact that most hip fractures occur indoors (1). The last hip fracture trial conducted in Oslo, based on nutrition intake, concluded that the intake of vitamin D in hip fracture patients in Oslo was adequate (117). Since then the adequate intake of vitamin D has been increased from 10 µg/day to 20 µg/day in the elderly. The level of 25(OH)D required for no PTH elevation is contradictive, but a level above 50nmol/L has been reported to have no effect on PTH secretion (112). It is suggested that 20 µg/day including sun exposure is sufficient to achieve 50 nmol/L in healthy adults (25).

Few studies have examined the two nutrients, vitamin K and 25(OH)D combined although it has been done (29;30). Both studies report low 25(OH)D and low vitamin K in hip fracture patients. The evidence that low vitamin K is associated with hip fracture is becoming increasingly convincing, however few studies has examined the association of a possible synergistic effect of the micronutrients in western populations. The two vitamins are thought to act synergistically upon osteocalsin production and activation. The latest intervention study investigated the effect of supplementing with a dairy product containing Ca, fortified with vitamin D alone or vitamin D and physiological doses of vitamin K combined, showed the largest effect on undercarboxylated osteocalsin and BMD for vitamin D and K combined (31). Furthermore, lower ucOC and improved BMD has been found after 1 year supplementation using physiological doses of vitamin D3, vitamin K1 and B6 in a healthy elderly population (118). This supports our findings of a synergistic effect between the two vitamins.

Low vitamin C has been linked to increased hip fracture risk mainly in prospective studies studying the intake of fruits and vegetables and in vitro studies. We have confirmed the association of low serum vitamin C in actual hip fracture patients compared with controls. A previous study of fruit and vegetable intake in Oslo showed a lower intake in a low-income area versus a high-income area (36) and hip fracture is reported to be more frequent in low-income areas (37). In our study, vitamin C concentrations in the patients were at the level of the low-income area, whereas the control group was at the level of the high-income area. A previous case control study in Oslo reported an association between low serum levels of vitamin C and hip fracture. However, in this study the blood was drawn postoperatively (119) and was probably hampered with increased inflammation as vitamin C in serum falls as a consequence of inflammation (120), as is the case under and after a hip fracture operation. We have confirmed the association when analyzing vitamin C preoperatively and adjusted for CRP in the multivariate analysis. Some authors suggest that the effect of low vitamin C is only seen in smokers (35;121). In our population only 18% of patients and 14% of healthy

controls were current smokers, a non-significant difference. Patients in our study took a greater number of medications. This may reflect a higher degree of illness and inflammation that may also cause increased oxidative stress and an increased need for antioxidants. The association of increased fracture risk in patients with low vitamin C and E remained, however, unchanged when adjusting for number of medications used and CRP.

The strongest correlation between vitamins and total osteocalsin was for vitamin C. In vitro cell culture studies supports bone growth, an increase in synthesis of collagen I, and osteocalcin in cells supplemented with vitamin C (122). Johnson et al recently reported that vitamin D and vitamin C synergistically increased the ostecalsin promoter activity in human renal cell carcinoma cells (123) supporting our data that vitamin C may act through osteocalsin. The reported protective effect of fruit and vegetables may be due to vitamin C's action on bone mineralization and may possibly add to the suggested antioxidant effect protecting against bone resorption as well as promoting collagen synthesis by vitamin C.

Review papers throughout the last decade suggests that high dietary intake of vitamin A is associated with increased fracture risk (53;124), but no dose-dependent increase in fracture risk after long-term exposure to high doses of retinol or beta-carotene has been found (125). The latest report from a large scale prospective study of elderly men and women in Norway found no evidence of an adverse effect of high serum retinol on hip fracture. If anything, there tended to be an increased risk at low retinol concentrations (52).

Barker et al found no evidence of skeletal harm associated with retinol exposure. The risk of any osteoporotic fracture, including hip fracture, was slightly less in the highest quartile. They also found a positive correlation between retinol and BMD, but found no relation between serum retinol and BALP or the bone resorption marker CTX (51). The discrepancy findings in BALP with our findings may be the study design as the study by Barker et al was a large scale prospective study. The mean serum retinol in the cases was at the same level as our control group. We examined serum retinol and BALP in actual hip fracture patients and found lower serum vitamin A levels compared with Barker et al.

In a national survey, good sources of vitamin A in Norway (in decreasing order) are: Vegetables (β-carotene), meat, cheese and butter spread due to fortification (54). 67% of the population aged 60 to 70 years report to take some form of dietary supplement and that the mean intake of vitamin A is adequate and near the recommendations of 900 Retinoic Acid Equivalents for men and 700 RAE for women. However, the serum retinol concentrations in the hip fracture patients in our study were low and in the lower end of the normal reference range. A majority had vitamin A deficiency or inadequate vitamin A status as defined by serum retinol < 0.70 μmol/L and serum retinol < 1.05 μmol/L respectively (53).

In summary, vitamin K1 is abundantly found in green leafy vegetables, but is also found in other vegetables, fruits and berries. The major dietary source of vitamin C is fruits, berries and vegetables, and the carotenoids found in vegetables are converted to vitamin A in the body. It has recently been confirmed that high intake of antioxidant rich foods containing vitamin C, E and β-carotene is associated with reduced risk of hip fracture in a Chinese population (126).

Based on our data and research of the literature low intake of fruits, vegetables and berries together with low intake of fatty fish and sun exposure may contribute in answering the question why the incidence of hip fracture is amongst the highest in the world in Oslo, Norway.

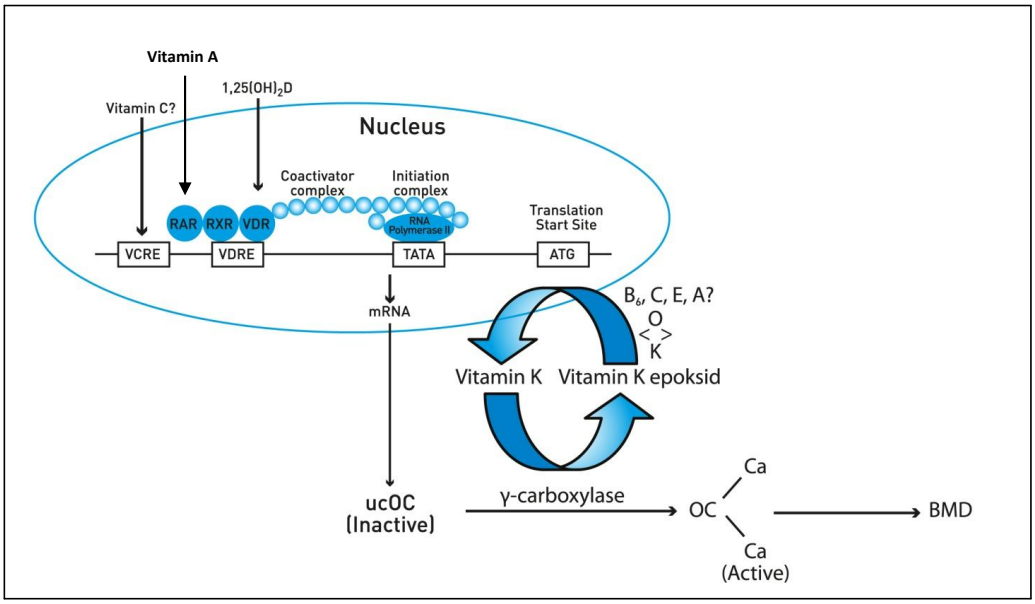
High homocysteine has been associated with increased risk of hip fracture(46). However studies are few. Unfortunately, we did not measure homocysteine or vitamin B2.

Hip fracture patients are often old and frail and these patients are commonly low in vitamin B. This knowledge has been known for decades and common multi vitamin B supplementation may be the reason why the B vitamins were not associated with increased risk of hip fracture. Out of the vitamins that Homocysteine depends on, vitamin B6 was in our study found to be the compromised vitamin and may increase Homocysteine. This needs further elucidation. Low serum vitamin B6 may be due to a poor food intake. Good sources of vitamin B6 are meat and products of meat, fruits and berries, bread, fish and fish products and dairy products in decreasing order (54). The foods listed above are also good sources for Ca, vitamin K, D, C, E and A. The Hordaland study found that intake of vitamin K1 and folate through green vegetables was independent risk factors for hip fracture (43;127). The association was based on food consumption data, not serum vitamin levels. We have confirmed the association for serum vitamin K1, but we did not find low serum folic acid in this study possibly due to common supplementation.

A possible synergistic effect between vitamins and the production and activation of osteocalsin:

BMD depends, amongst other things, on the amount of calcium retained in bone. Retention of Ca in bone requires activated matrix osteocalsin. Based on the literature and our findings we have suggested a mechanism of interaction between nutrients and ability to mineralize bone, (**figure 11).** The mechanisms are highly suggestive and needs further elucidation.

Figure 11: Suggested mechanism for how micronutrients may interact on osteocalsin



OC= Osteocalsin, RAR = retinoic acid receptor, RXR = receptor X receptor, ucOC= undercarboxylated osteocalsin, VDR= vitamin D receptor, VCRE = vitamin C responsive element, VDRE = vitamin D responsive element.

The bonespecific protein osteocalcin binds calsium to bone but osteocalcin is formed in an inactive form, undercarboxylated osteocalcin, ucOC. Vitamin K acts as a catalyst in the activation to form carboxylated osteocalcin, the active form. In the process a vitamin K epoxide is formed. Vitamin K may be regenerated by vitamin B6 and possibly vitamin A, C or E.

The promoter for the prepeptide ucOC contains both a vitamin D and a vitamin C responsive element that may regulate ucOC. It is also possible that vitamin A could regulate the promoter for the ucOC gene as the retinoic acid receptor forms heterodimers with RXR (27;53;123;128-132).

Even though vitamin D in combination with Ca supplementation prevents bone loss, and increase BMD (133-135), the meta analysis by Bolland MJ et al found only a marginally fracture risk reduction after supplementation with vitamin D and Ca (133). The reason for the marginal finding may be that other vitamins and protein energy may also be important.

A recent review of nutrients associated with bone health indicates that vitamin D and Ca supplementation is insufficient for optimizing bone health as vitamin B6, C and K may also play an important role (136). Our data adds vitamin E and A to the list.

5.2 The effect of nutritional supplementation of hip fracture patients on boneturnover markers, paper III

The reported positive effect of vitamin D, Ca and vitamin K upon bone tissue has been demonstrated in healthy, pre- and postmenopasal as well as osteoporotic elderly populations (28;133;134;137). To the best of our knowledge, the effect of Vitamin D, Ca and vitamin K1 on BTM in actual hip fracture patients has not previously been studied.

There are many reasons why there was no effect on BTM after 4 months supplementation. The effect of nutrition supplementation might have been masked by the strong increase in BTM signal after fracture, as elevated BTM have been described to last for several months, up to a year after fracture (71) and the short period of 4 months supplementation may have been too short. Another reason may be that the supplementation of energy and protein might have been insufficient. Most nutritional interventions after fracture have focused on increasing protein and caloric intake through nutrition drinks with mixed results (138;139). We encouraged oral nutrition drinks and gave individual advice on how to improve protein and energy. However, we were not able to prevent weight loss, commonly reported after hip fracture (140). A negative energy and protein balance may have counteracted any beneficial effect of the micronutrients. Poor compliance to oral liquid supplements in hip fracture patients has been reported by others (100). We can also discuss if the dosage provided was large enough. We used supplements commonly used in elderly (cod liver oil) combined with a Ca, vitamin K1 and D3 containing tablet to achive the recommended intake of vitamin D and K1. However, the recommended intake of vitamin D is contradicted as well as for vitamin K1. The recommended daily dosage of vitamin K1 in the USA is 95 µg. The literature suggests, however, that 200-500 mg is required to achieve an optimal carboxylation of osteocalsin (141). It has been suggested that pharmacological doses of vitamin D rather than physiological doses is required to enhance 25(OH)D from a deficient state at 50 nmol/L to an adequate state at 75 nmol /L (137). However, lower ucOC and improved BMD has been found after 1 year supplementation using physiological doses of vitamin D3, vitamin K1 and B6 in a healthy elderly population (118). In a post fracture state, higher dosages may be necessary. The recommended adequate level of 25(OH)D is controversial and one can speculate if we achieved adequate serum 25(OH)D. Mean 25(OH)D was 61 nmol/L in the intervention group and 45 in the control group. The suggestion that 50nmol/L indicates vitamin D deficiency and that 75 nmol/L is required for optimal bone health (142) indicates that even though there was a statistical difference between the two groups, this may not have been enough to induce clinical differences measured by BTM as 25(OH)D was below 75 nmol/L in both groups. It is also possible that some controls may have taken the supplement producing a false negative response. At 4 months follow up, vitamin K1 status had improved in the control group as well as in the intervention group. Some patients in the control group may have received supplement since control patients for ethical reasons were not told not to take supplements and were admitted to the same rehabilitation institutions as the controls. This is supported by the fact that 16% of the control subjects expressed a 4 fold increase in vitamin K1 from baseline to 4 months follow up. As vitamin K1 is mainly found in green leafy vegetables, the increase is most likely due to supplementation as it is unlikely that the hip fracture patients suddenly has started to eat more green leafy vegetables after fracture.

Even though vitamin supplementation in the present study did not achieve differences in BTM, vitamin supplementation may still have been warranted as an increase in all cause

mortality has been found in patients with 25(OH)D below 50 – 60 nmol/L, (143-145). A further decrease in 25(OH)D seems to have been prevented in the supplementation group compared with controls. A low 25(OH)D after fracture may increase the risk of recurrent falls and thus a second fracture (133). It has also been suggested that inflammation contributes to prolonged rehabilitation after hip fracture (146), and a low 25(OH)D has recently been associated with increased inflammation (94). We have previously reported better mobility in home dwelling patients admitted to the intervention group in the OOT 4 months after fracture compared with home dwelling patients in the control group (23). The improved 25(OH)D status in the intervention group may have contributed to this as vitamin D is associated with muscle strength (133;147).

5.3 Is specific micronutrient deficiency related to increased risk for Delirium? Paper IV

To our knowledge, this is the first study to report lower 25(OH)D in hip fracture patients who experienced a delirium compared with patients who did not experience a delirium throughout the hospital stay. We also tested the association using 25(OH)D = 75 nmol/L as cut off for sufficiency as this recently has been recognized as cut off for maintaining good cognition (148;149). Our model does not support the higher cut point for 25(OH)D, possibly due to a large confidence interval and lack of statistical power. However, this is only the case for acute delirium, for other reasons that higher cut point might be relevant (at least for cognitive decline).

Vitamin D receptors has been located in the human cortex and hippocampus, which are key areas for cognition and may regulate neurotransmission, neuroprotection and neuro immuno modulation including anti- inflammatory and antioxidant effects (90-92). This may explain our findings for vitamin D. A cross sectional study examined 25(OH)D in patients who experienced a delirium found a common hypovitaminosis D in delirious patients (150), and a pilot study of intensive care unit patients showed that low vitamin D was associated with delirium at borderline significance (148). However, Lapid et al studied hypovitaminosis D in a geriatric psychiatric ward and found no association between vitamin D and cognitive function including delirium, however only n= 13 patients with delirium were presented in this study (151).

Our findings accords well with the pathospysiologcal theory of increased inflammation in delirious patients and that delirium is a consequence of reduced anti – inflammatory activity (93) even though CRP was not associated with increased risk of delirium. No difference in CRP has also been found by others, and it is suggested that pro inflammatory cytokines in cerebral spinal fluid and neuron inflammation may differ from systemic inflammation measured by CRP (93). Vitamin D has been inversely associated with inflammation and chronic inflammation is closely associated with oxidative stress that activates inflammatory response proteins (152). Cytokine and interleukin differences have been reported, in Cerebra Spinal Fluid (CSF) from hip fracture patients with mild cognitive impairment who experienced a postoperative delirium compared with patients who remained lucid (93). Morandi et al discuss that the association between low 25(OH)D may be through vitamin D's potential to down regulate the production of tumor necrosis factor α, IL6 and nitric oxide in vitro (148).

Lundstrøm et al demonstrated a decrease in the delirium incidence in hip fracture patients after treatment in a specialized geriatric ward compared with a traditional orthopedic ward.

The multi-intervention included protein enriched meals (85;153) and our research group has previously reported that low BMI was associated with risk of delirium (13).

5.4 Methodological considerations:

Vitamin A, B1, B2, B6, C and 25(OH)D in serum fall as a consequence of inflammation (120;154), as is the case under and after a hip fracture operation. The blood tests were therefore performed as soon as possible after informed consent was given and prior to surgery and we adjusted for CRP in the multivariate regression model. Due to the strict requirement that blood had to be taken preoperatively, we only included 116 out of 216 possible candidates. All 216 patients underwent pre surgical blood test and there was no difference in age or markers of acute or chronic illness such as CRP, Fe, creatinin or Albumin between those enrolled for vitamin and bone turnover tests and those not enrolled.

Furthermore, we wanted to elucidate if low vitamins reflected a pre fracture risk association for delirium. However we measured the incidence of delirium throughout the hospital stay and the expected fall in serum vitamins due to inflammation may contribute to delirium postoperatively also in individuals adequate in vitamins preoperatively. This aspect needs further elucidation before supplementation studies are designed.

We measured body weight on the first possible day after operation and vitamins preoperatively. Therefore, we anticipate that the nutrition parameters are as close to pre fracture state as possible. However, it should be acknowledged that BMI is imprecise in this population as height was measured using standing height in the controls and knee height was measured in some (n=29) patients unable to stand. Data from the OOT (155) indicates that knee height underestimates height and may have contributed to higher BMI in patients. Furthermore, it is well known that stature declines with age, but standing height was used as a preferred measurement in patients as controls were measured using standing height in order to use the same methodology when possible. Standing height is the preferred measurement in the Mini Nutritional Assessment, NRS2000 and the Malnutrition Universal Screening Tool, screening tools for malnutrition and was therefore chosen as standard measurement. We do not have data on weight loss prior to fracture; a measure that perhaps indicated nutritional risk better than BMI, but weight loss was impossible to obtain due to cognitive impairment in this patient population.

We had insufficiently detailed data on comorbidity in the controls, and we were thus unable to adjust for some known risk factors for hip fracture like diabetes, chronic kidney disease, usage of antidepressants, warfarin, sleeping pills, or anti resorptive agents and hence limits the interpretation of our data. We counteracted this to the best of our ability by adjusting for the number of prescription drugs taken and CRP as a proxy measure for inflammation.

It is uncertain whether the fracture itself could have induced change in bone turnover towards resorption (156;157). However, it has been reported no difference in BTM measured shortly after fracture (within 2 days) compared with pre fracture data (71). This indicates that BTM measured within hours of hip fracture reflects pre fracture levels. Osteocalsin and CTX1 express a nocturnal peak, however not found in postmenopausal women (69), but blood should possibly have been standardized to early morning samples. We were unable to perform such standardization due to the priority of operation as soon as possible and lack of project staff. However, when possible, the blood was sampled in the early morning for assays of all vitamins and BTM. In controls, blood was drawn in the early morning after an overnight fast.

Large differences in serum 25(OH)D has been reported in serum from the same individuals depending on the laboratory and assay used (25;158). The highest values, and most reliable measures, have been reported using High Performance Liquid Cromatography, followed by Radio Immuno Assay. We used Radio Immuno Assay. In a study, using 50 nmol/L as sufficient 25(OH)D, only 8 % of patients were regarded as deficient using High Performance Liquid Cromatography compared with 22% using RadioImmuno Assay and 43% using chemiluminescent immunoassay (158). However, we used the same assay for all patients and controls. The "*Vitamin D Standardization Program"* has developed protocols for standardizing existing serum 25(OH)D data from national surveys around the world. 25(OH)D samples run at the hormone laboratory, Aker Norway and at the vitamin D Standardization program show a 96% correlation (159).

A small increase in erythrocyte B6 has been shown during acute inflammation (157). The authors suggest that the results reflect a redistribution of B6 from plasma to erythrocytes during acute inflammation. We therefore performed the vitamin B6 analysis in serum and in erythrocytes in patients. Unfortunately, vitamin B6 was measured in serum only in the controls as this knowledge was unknown to us in 2005.

The method for fullblood vitamin B1 had changed from 2005 when the control samples were collected to 2009 - 2011, the time point when the samples for the patients were collected. Hence, vitamin B1 between patients and controls was not comparable. We also did not have data for vitamin B2 or Homocysteine. This is unfortunate. The vitamin B2 was omitted as the method available at the Nutrition Laboratory, Aker, was unsuitable (personal communication Thomas Bøhmer) and Homocysteine samples in controls were lost due to technical error of the freezer during summer 2010.

We did not measure serum retinyl esters, a better measure for high vitamin A intake (53). This was because we wanted to test the hypothesis that low, not high, serum retinol might be associated with increased hip fracture risk. Also, the total amount of blood to be drawn in each patient would have been unwarrantably high if we included all assays of our wishes; and in excess vitamin A consumers, serum retinol is elevated as well as retinyl esters. We therefore limited our analysis to serum vitamin A.

We did not undertake a dietary survey due to the fact that as many as 50% of hip fracture patients are cognitively impaired during the acute phase of hip fracture (13). We also considered the possibility of contaminating the nutrition intervention to the control group if a clinical dietician entered the orthopaedic ward daily and a dietary history could therefore only be obtained in the intervention group and was therefore omitted.

One could also discuss if we included only the healthiest home dwelling subjects as controls. The controls were drawn randomly from inhabitants of the appropriate age range, but we cannot rule out this possibility.

In carrying out the pre operative intervention, we aimed for no more fasting than 6 hours from solid foods and 2 hours from clear drinks. Fasting time was unfortunately not recorded, but regarded as standard protocol for treatment in the intervention patients at the acute geriatric ward. However, this aspect of the intervention was one of the major challenges due to the fact that the nurses at the acute geriatric ward did not know when exactly the patients were scheduled for operation (due to constant change in scheduled operation time). This uncertainty created worries amongst nursing staff due to the assumed risk of respiratory pneumonia if meals are ingested shortly prior to operation. This worry was probably unwarranted as studies have shown no increased risk of respiratory pneumonia in non fasting

surgical patients versus fasting surgical patients probably due to new methods for anesthesia (160-162). However, the worry persisted throughout the study.

A nutritionist assessed the individual patients need for individualized meals during the hospital stay. However, the ward was often overcrowded (163) and even if an individual meal plan was given it is uncertain whether it was followed. Upon discharge, the patients received personal nutrition advice in their summary notes, but no follow up of the intervention was performed due to the fact that it was the outcome of treatment between the wards that was the primary endpoint in the OOT.

We also experienced difficulties in weighing the patients. BMI was missing in a large number of patients at baseline, n= 41.This was probably mainly due to early discharge especially from the orthopedic ward, but also due to the fact that we only had one chair scale situated in the acute geriatric ward. This had to be transported to the orthopedic ward for each hip fracture patient to be weighed. We also experienced difficulties in weighing the patients at 4 months follow up as this was performed in the patient's own home. We only transported a portable standing scale and as not all patients were able to stand long enough on the scale, weight was missing.

In order to comply with the need for increased need for protein energy, we also gave individual advice as how to improve food intake and recommended protein enriched oral nutrition drinks daily. Despite our efforts, patients in both groups lost weigh possibly due to the lack of follow up during the 4 months follow up.

5.5 Implications for the patient and the society

The prevalence of malnutrition in Norwegian nursing homes is high, perhaps as high as 60%, dependent on the method used to detect malnutrition (164). In this thesis we have detected general malnutrition to be associated with risk of hip fracture, low 25(OH)D to be associated with delirium in hip fracture patients and that vitamin D supplementation may have contributed in prevention of a further decline in 25(OH)D after fracture. Illness like depression, dementia, chewing, swallowing, eating disorders and somatic illness may contribute to malnutrition, but also a lack of knowledge in medical nutrition treatment and time amongst healthcare workers may contribute (165;166). Several reports reveal failing procedures for nutrition practise (164;165;167-169). In a questionnaire survey amongst several thousand medical doctors and nurses in Scandinavia found that Norway had the poorest routines for nutrition practice in hospitals despite the high prevalence and severity of malnutrition (165). In 570 Norwegian nursing homes, only 16 % had written procedures for nutrition practice. Less than half reported to weigh the patients upon admission, and even fewer institutions reported to weigh the patients regularly. The survey also found that the reason for failure in nutrition procedures was lack of knowledge in clinical nutrition (165). In a national survey, 80 % of healthcare workers in the home help service for elderly wants more knowledge in clinical nutrition (170).

Malnutrition is associated with a number of medical conditions and diseases, amongst those osteoporotic fractures, infectious disease, and cognitive decline (13;107;171-174). Hip fracture results from prolonged untreated osteoporosis and a fall. Approximately one third of community dwelling persons, above 65 years and older, experience at least one fall incidence

annually. The incidence goes up by 50 % in those aged > 80 years and is at serious risk of major injury like hip fracture with increased morbidity and mortality (175). The incidence of osteoporotic fractures in Norway, measured by hip fractures, is amongst the highest in the world (1). Osteoporotic fractures are not only detrimental for the patients, but are also a great burden for the society. The trauma caused by fracture severely inhibits the patients both physically and mentally increasing the need for help from the healthcare service as fractures as well as infections are some of the factors frequently associated with delirium that possibly initiates the onset of dementia and or accelerates the disease (176).

Disease related malnutrition increases the risk for complications, reduces infection resistance, physical and cognitive function that compromises quality of life, delays recon valence and is related to increased mortality (164). A recent study of 250 000 community –dwelling Danes found an increased risk of all-cause mortality in patients with 25(OH)D below 50-60 nmol/L (143). This has also been found by others (144). Our hip fracture patients achieved 60 nmol/L 25(OH)D after 4 months supplementation, however in the control subjects 25(OH)D had declined to 45 nmol/L. Even if 25(OH)D din not improve bone turnover markers, vitamin D supplementation may still have been warranted. No vitamin D supplementation trials has been conducted with deaths as a primary outcome (57).

The results of nutrition supplementation trials are conflicting (101;138;177) possibly due to poorly designed trials, poor compliance, that the patients has a need for nutrition care at several levels, poor routines for nutrition and / or lack of knowledge amongst healthcare professionals. The latest meta analysis found no statistical beneficial effect of oral nutrition feeds after hip fracture on mortality although the intervention group was in favor and nutrition supplements may possibly reduce unfavorable outcomes (139). It has been found that dietetic assistance during hospitalization induced lower mortality 4 months after hip fracture compared with usual care and patient satisfaction was significantly greater in the intervention group at discharge (178).

Hospitalized patients above 75 years have on average 3 diagnoses. Of these, 25 percent has 6 diagnoses. Many has nutrition related problems. Patients in need for nutrition treatment may not get adequate treatment as there is a large gap between the need for help and the care given(179).

From 2012 the Norwegian government decided that all patients should be treated nearest to home when possible (“samhandlingsreformen”). The local counties should at a greater extent treat all patients in near collaboration with the specialist hospital care. The need for knowledge in clinical nutrition in nursing homes and in the primary care of home dwelling patients is therefore increased in order to prevent fractures and rehabilitate well.

The potential cost benefit of nutrition intervention:

The Directorate of health indicates that clinical nutrition treatment can be carried out by non nutrition specialized healthcare professionals in close collaboration with an authorized clinical dietitian (179). More recently, a study from the Nederland show that the excessive costs of treating malnourished patients in nursing homes is 1,5- 2 times as expensive compared with well nourished patients (180).This bird perspective of malnutrition in hip fracture patients implies that specialist nutrition knowledge in nursing homes and in the home help service for the elderly is beneficial in order to improve quality of life in the elderly and reduce the health care costs in the elderly. These assumptions should be tested and documented in future studies.

6. Conclusions

Hip fracture is a traumatic event with dramatic short and long term consequences for the patients and next of kin. Malnutrition is common in this patient group and malnutrition may increase risk of hip fracture.

We found that patients with low micronutrients: vitamin K1, 25(OH)D, vitamin C, E and vitamin A were at increased risk of hip fracture independent of BMI. However, vitamin D3, K1 and Ca supplementation did not improve bone turnover markers. Hip fracture patients low in 25(OH)D may be at increased risk of experiencing a delirium during the hospital stay.

7. Recommendations for future research

It is widely believed that malnutrition is associated with increased risk of hip fracture and that malnutrition increased risk of complications after trauma. However, the question as to whether nutrition intervention can prevent and improve the outcome after hip fracture remains unsolved. Good nutrition interventions should provide not only vitamin D and Ca, but should also supplement with vitamin K1, vitamin C, E and A as well as provide adequate protein and energy in order to study a change in improvement of hip fracture prevention and recovery.

Secondly, it should be tested if securing adequate 25(OH)D can possibly reduce the incidence of delirium during hip fracture treatment and study if the expected fall in micronutrients during acute phase of hip fracture contribute in increased risk of hip fracture during the hospital stay.

The suggested studies are costly and demanding, but given the increase in age in our population, and an increase in number of people achieving an age above 80 years, the magnitude of the problem arising from hip fracture is growing. Therefore, it should be possible to get funding for such studies through applications.

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Ref Type: Generic

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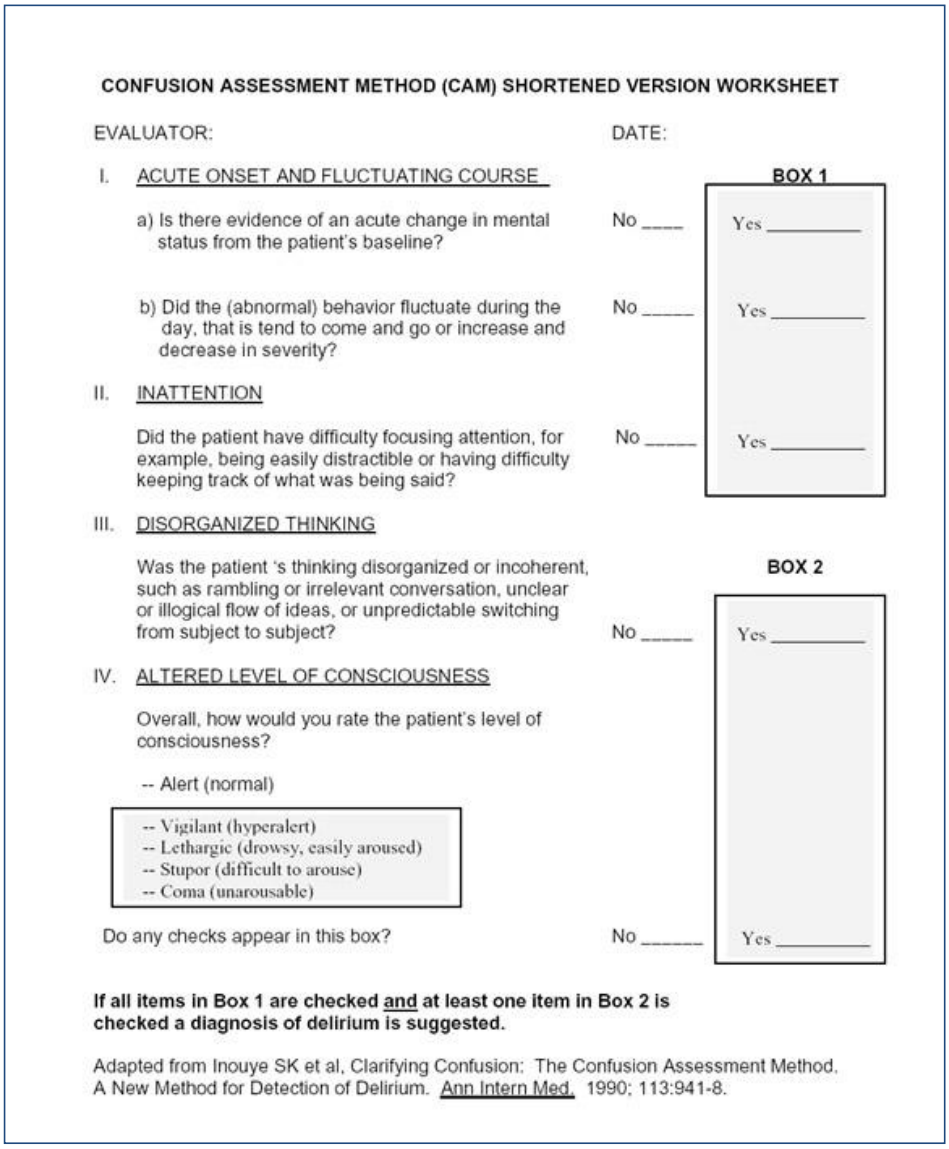
Appendix 1: The Confusion Assessment Method form



CONFUSION ASSESSMENT METHOD (CAM) SHORTENED VERSION WORKSHEET

EVALUATOR: DATE:

I. ACUTE ONSET AND FLUCTUATING COURSE

a) Is there evidence of an acute change in mental status from the patient's baseline? No ____ Yes _____

b) Did the (abnormal) behavior fluctuate during the day, that is tend to come and go or increase and decrease in severity? No _____ Yes _____

II. INATTENTION

Did the patient have difficulty focusing attention, for example, being easily distractible or having difficulty keeping track of what was being said? No _____ Yes _____

BOX 1

III. DISORGANIZED THINKING

Was the patient 's thinking disorganized or incoherent, such as rambling or irrelevant conversation, unclear or illogical flow of ideas, or unpredictable switching from subject to subject? No _____ Yes _____

IV. ALTERED LEVEL OF CONSCIOUSNESS

Overall, how would you rate the patient's level of consciousness?

-- Alert (normal)

-- Vigilant (hyperalert)
-- Lethargic (drowsy, easily aroused)
-- Stupor (difficult to arouse)
-- Coma (unarousable)

Do any checks appear in this box? No _____ Yes _____

BOX 2

If all items in Box 1 are checked and at least one item in Box 2 is checked a diagnosis of delirium is suggested.

Adapted from Inouye SK et al, Clarifying Confusion: The Confusion Assessment Method. A New Method for Detection of Delirium. Ann Intern Med. 1990; 113:941-8.

Appendix 2: Flowchart of patients

Figure 1: Flow chart of the patients, paper I,II and III

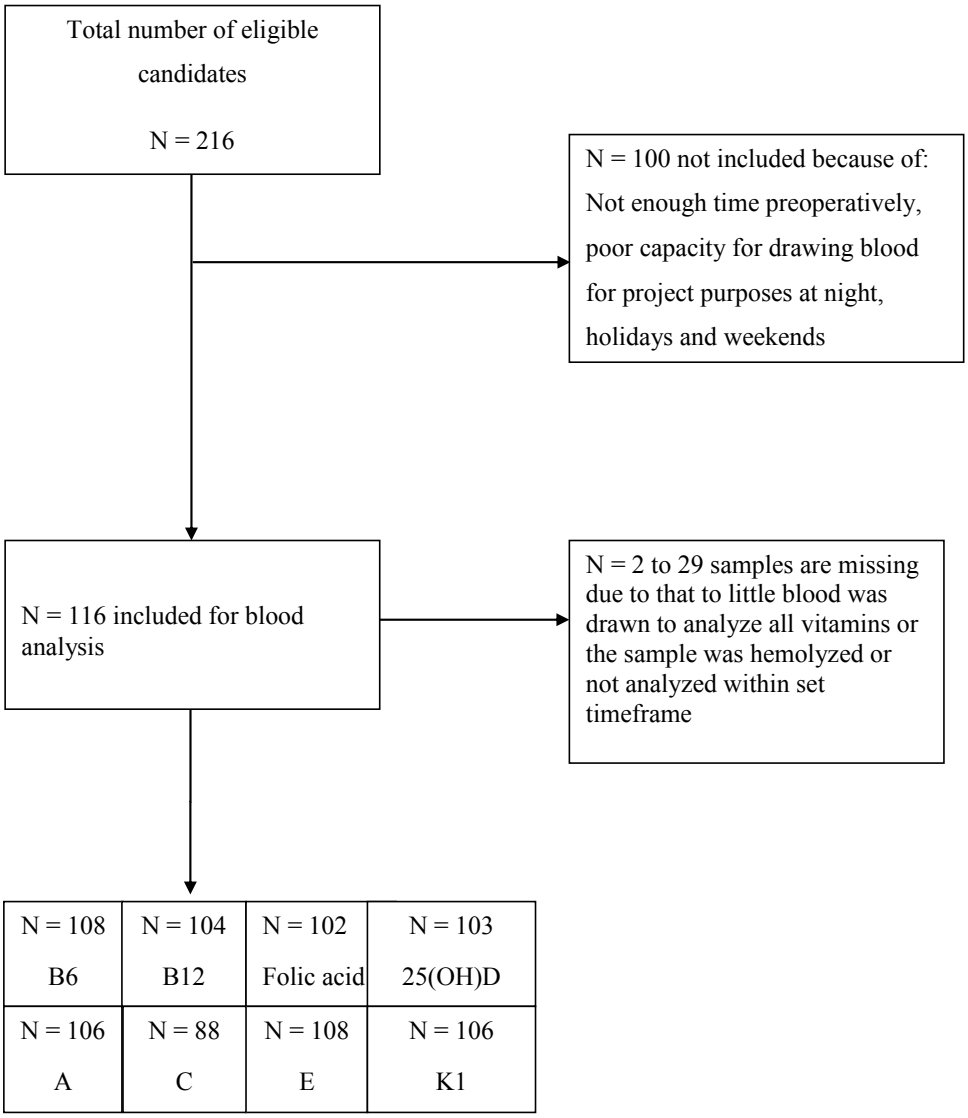
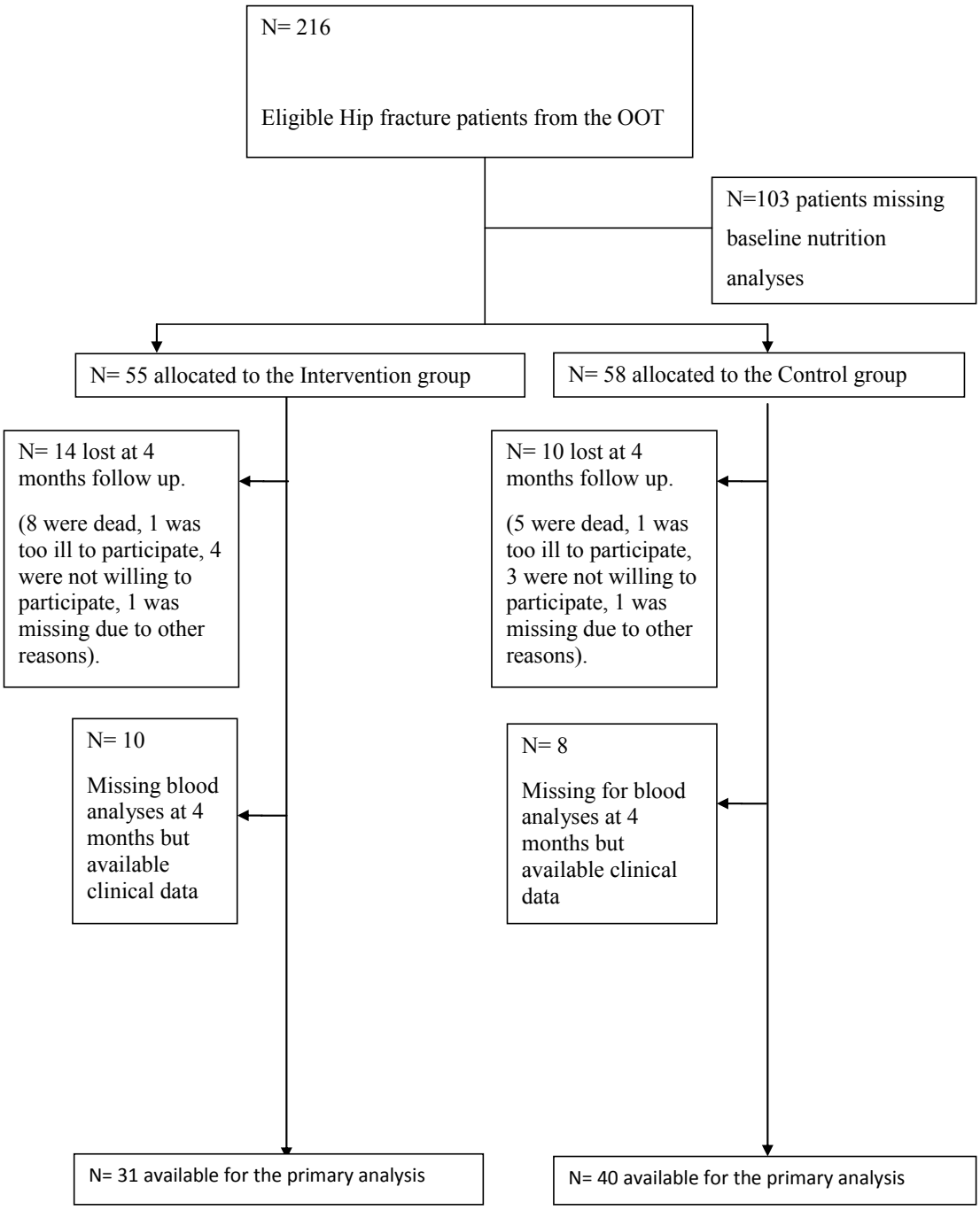


Figure2. Patient flow, paper IV



Papers I-IV:

II

IV