Dietary predictors of bone mineral density, hip fractures, mobility and muscle strength

- in community-dwelling older persons

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Scientific environment

This project was conducted from September 2013 to July 2018 at the Department of Clinical Science, Faculty of Medicine, University of Bergen. The main supervisor was Professor Jutta Dierkes and the co-supervisors were Dr Therese Karlsson, Professor Anette Hylen Ranhoff and Professor Grethe Seppola Tell.

The current project is part of a larger research initiative investigating metabolic and mental health effects of fish consumption called Fish Intervention Studies (FINS). The Norwegian Seafood Research Fund (FHF) funded the project (grant number 900842).

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Abbreviations

ALM	Appendicular lean mass
BIA	Bioelectrical impedance analysis
BMD	Bone mineral density
BW	Body weight
DXA	Dual-energy x-ray absorptiometry
DHA	Docosahexaenoic acid
EWGSOP	The European Working Group on Sarcopenia in Older people
FGF23	Fibroblast growth factor 23
FFQ	Food frequency questionnaire
HGS	Hand grip strength
HUSK	The Hordaland Health Study
IOM	The Institute of Medicine
NMS	New Mobility Score
NNR5	Nordic Nutritional Recommendations 2012
PTH	Parathyroid hormone
RANK	Receptor activator of nuclear factor $\kappa\beta$
RANKL	Receptor activator of nuclear factor $\kappa\beta$ ligand
RCT	Randomized Clinical Trial

RDA	Recommended Daily Allowance
SD	Standard deviation
SMI	Skeletal muscle index
TUG	Timed-up-and-go test
UVB	Ultraviolet radiation B
VDR	Vitamin D receptor
WHO	World Health Organization
n3 PUFAs	n3 polyunsaturated fatty acids
1,25-$(OH)_2$D	1,25-dihydroxyvitamin D (calcitriol)
25-(OH)D	25-hydoxyvitamin D (calcidiol)
24HR	24 hour recall

Abstract

Background: The aging process involves decline of both muscle and bone mass. The loss of muscle mass is associated with decreased muscle strength and mobility, while loss of bone mass leads to osteoporosis and increased risk of fractures. This process maybe accelerated due to poor dietary quality, low energy intake and undernutrition. Fish is a good source of nutrients that are associated with preserving both muscle and bone mass. In particular, fish is contributing substantially to vitamin D intake.

Overall aim: The overall aim of this PhD-project was to investigate dietary predictors of bone mineral density, risk of hip fractures, muscle strength and mobility in community-dwelling older persons.

Specific aims:

1. The aim was to summarize the available literature in a systematic literature search on randomized clinical trials investigating the effects of vitamin D supplementation (with or without calcium supplementation) on muscle strength and mobility in community-dwelling older persons, presented as a narrative systematic review and a formal meta-analysis on measures of muscle strength and mobility.
2. The aim was to examine the association of fish intake with bone mineral density and risk of hip fractures in the Hordaland Health Study (HUSK), by conducting
   a) a cross sectional analysis of the association between fish intake and bone mineral density in middle - aged and older participants, and
   b) a prospective analysis of the association of baseline fish intake with the risk of hip fractures in the older cohort during a follow -up of ten years.
3. The aim was to conduct a clinical observational study on weight change and mobility in community-dwelling hip fracture patients during the first two to three months after the fracture.

Results:

1. The systematic review included 15 studies. In the majority of studies, no improvements in muscle strength or mobility were observed after administration of vitamin D with or without calcium supplementation. The quantitative meta-analysis did not reveal any clinically relevant effects of vitamin D supplementation neither on handgrip strength (seven studies) nor on the timed-up-and-go test (five studies).

2. a) Cross-sectional analyses from the HUSK study showed an association between high fish intake and higher bone mineral density in older women, but not in middle-aged women or in men.
   b) A low intake of fish was associated with increased risk of hip fracture in older men during a follow-up of ten years. This association was not evident in older women.

3. Among community-dwelling hip fracture patients with normal cognitive function, age and weight loss were associated with insufficient mobility two months after the fracture.

Conclusions: Vitamin D supplementation had no clinically relevant effect on measures of muscle strength and mobility. Fish intake did not have consistent effects on bone mineral density and risk of hip fractures. The findings of positive effects of fish intake in subgroups but not in the entire population have to be interpreted with care. Age and weight loss were the main determinants of mobility in patients after a hip fracture. In conclusion, maintenance of weight, muscle mass and bone mineral density are key issues for health of older persons. The results of the present investigations suggest that the there is a need for more studies regarding diet and health outcomes in community-dwelling older persons.

List of publications

I. Rosendahl-Riise H, Spielau U, Ranhoff AH, Gudbrandsen OA, Dierkes J. *Vitamin D supplementation and its influence on muscle strength and mobility in community-dwelling older persons: a systematic review and meta-analysis.* J Hum Nutr Diet. 2017;30(1):3-15. Epub 2016/07/28.

II. Rosendahl-Riise H, Karlsson T, Drevon CA, Apalset EM, Nygård OK, Tell GS, Dierkes J. *Total and lean fish intake is positively associated with bone mineral density in older women in the community-based Hordaland Health Study.* European Journal of Nutrition. 2018. Epub 2018/03/15

III. Rosendahl-Riise H, Sulo G, Karlsson T, Drevon CA, Dierkes J, Tell GS. *Limited benefit of fish consumption on risk of hip fracture among men in the community-based Hordaland Health Study.* Nutrients. 2018. Epub 2018/07/06.

IV. Dierkes J, Rosendahl-Riise H, Ådnanes S, Skodvin VA, Strand ES, Ranhoff AH. *Weight changes and mobility in the early phase after hip fracture in community-dwelling older persons.* Submitted to Journal of Nutrition, Health and Aging June 23 2018

Contents

1. Introduction

1.1 Aging – loss of muscle and bone mass

1.1.1 Aging

Ever since the early Greek and Roman history, aging has been a problem for humanity. The ancient Greeks, represented by Aristoteles (384 BC), actually "abhorred aging, since it represented corruption of their highly prized youthful vigor and was considered as a chronic, incurable, and progressive disease". The widespread assumption was thus that old men should not engage in any activity because of their frailty. Cicero (44 BC), however, argued that: "it is our duty to resist old age, to compensate for its defects, to fight against it as we would fight a disease, to adopt a regimen of health, to practice moderate exercise; and to take just enough food and drink to restore our strength". The Greek physician and philosopher Galen of Pergamon (129 AD) saw aging as a stage between illness and health. He emphasized the importance of nutrition and exercise for healthy aging (1). Today, the goal is still healthy aging, and the World Health Organization (WHO) (2) has defined healthy aging as: "the process of developing and maintaining the functional ability that enables wellbeing in older age". The present thesis will focus on three aspects that are a part of aging: sarcopenia, osteoporosis, and hip fractures. The nutritional factors that may be important in preventing and managing these aspects will be outlined and discussed.

There is no conclusive definition of an older person, but a cutoff point of 65 years is often used. The average life expectancy has risen dramatically in the last few decades, owing both to a dramatic decrease in infant mortality in the developing countries and to better treatment of diseases associated with aging. In particular, there has been a marked increase in life expectancy above the age of 60. This means that an increasing number of people can expect to live to the age of 60 or longer, and more people will reach an age of 80 or older (2). The world's population older than 65 years is expected to double from 2010 to 2040, from about 506 million to 1.3 billion

(3). The same trend is evident in Norway, where the number of older persons (aged above 67) is anticipated to double over the next decade (4). In 2015, 125 million people worldwide were 80 or older, and the oldest age group is estimated to increase dramatically by 2050 (5). This growing elderly population will make increasing demands on health services. There is also an urgent need for measures to improve quality of life and to prevent conditions that are frequent in older persons.

With increasing age there are several changes taking place in the body, occurring on molecular, cellular, and systemic levels (**Table 1**). The molecular and cellular changes are beyond the scope of this thesis and have been summarized elsewhere (6).

Table 1: Physiological changes having an impact on the nutritional status and physical function in the course of aging (inspired by Table 9.1 in (7)).

Organ or system	Degenerative characteristics
Skeletal muscle	Muscle mass and function decline with advancing age (sarcopenia), owing to reduced muscle contractility and reduced levels of sex hormones. This leads to an increased risk of falls and fractures.
Fat mass	Fat mass increases during aging. Changes in body composition affect the energy expenditure.
Mouth and gastrointestinal tract	Poor dental status may lead to difficulties with chewing food. Reduced saliva production may lead to difficulties with swallowing food and affects taste and smell. Reduced production of stomach acid affects the bioavailability of several nutrients, e.g. vitamin B_{12}. In the small intestine, bacterial overgrowth may affect absorption of nutrients. The colon loses its motility, which may lead to constipation and diverticular disease.
Immune system	The skin barrier (passive immune system) and the cellular immune system become compromised with increasing age, which means an increased risk of infections and hospitalizations.
Lungs	Reduced number of pulmonary alveoli may lead to reduced exercise capacity.
Kidneys	A reduction in the number of nephrons may lead to increased blood pressure and problems with fluid and electrolyte balance.
Brain	Dementia and cognitive decline have a major impact on every aspect of life, including the nutritional status (e.g. remembering to eat or how to eat).

Aging is associated with changes in body composition and function. In particular, muscle mass, strength, and mobility are declining, a process that is known as sarcopenia. The decline in bone mass eventually leads to osteoporosis (low bone mineral density), a condition that increases the risk of fractures. In what follows, attention will be focused on these two conditions in addition to hip fractures, the most severe consequence of osteoporosis.

1.1.2 Muscle mass and strength, physical function and sarcopenia

The term "sarcopenia" was first used to describe the age-dependent decline in muscle mass by Rosenberg in 1989 (8). It is derived from the Greek *sarx* = flesh and *penia* = loss. Prior to 2011, sarcopenia was only defined as loss of muscle mass (9), but later it was redefined by the European Working Group on Sarcopenia in Older people (EWGSOP) (10) as "a syndrome characterized by progressive and generalized loss of skeletal muscle mass and strength with a risk of adverse outcomes such as physical disability, poor quality of life and death". Sarcopenia is diagnosed on the basis of predefined diagnostic criteria. These are 1) a low muscle mass and 2) either low muscle strength or poor muscle performance. The methods used for measuring muscle mass, strength, and performance according to the EWGSOP are listed in **Table 2**. This means that the modern definition of sarcopenia includes not only loss of muscle mass, but also loss of strength. However, the definitions and the criteria still vary (10-12). In the US, for example, the definition formulated by the International Working Group on Sarcopenia (13): "the age-associated loss of skeletal muscle mass and function" is preferred.

Table 2: Methods for measuring muscle mass, strength, and performance according to EWGSOP. Modified after (10).

Variable	Method	Clinical practice
Muscle mass	Computed tomography (CT) Magnetic resonance imaging (MRI) Dual energy X-ray absorptiometry (DXA) Bioelectrical impedance analysis (BIA) Total or partial body potassium in fat-free soft tissue	BIA DXA Anthropometry
Muscle strength	Hand grip strength Knee flexion/extension Peak expiratory flow	Hand grip strength
Physical performance	Short Physical Performance Battery (SPPB) Usual gait speed Timed get-up-and-go test Stair climb power test	SPPB Usual gait speed Get-up-and-go test

In 2016, sarcopenia received its own diagnostic code, ICD10-CM code M62.84, which means that it is now recognized as an independent, geriatric condition. To satisfy this code, one has to measure muscle mass by dual energy X-ray absorptiometry (DXA), perform a 10-meter gait speed and/or a hand grip strength measurement, or use a simple, validated questionnaire called SARC-F using predefined cutoffs (14, 15).

At present, screening for sarcopenia is not part of the everyday routine either in community-dwelling older persons or in the nursing homes or hospitals. The prevalence of sarcopenia is therefore unclear, but it is estimated to be 5-13% in 60-70 age group, and 11-50% in persons over 80 (16). In a study using the definition proposed by EWGSOP (10), the prevalence was found to be 1-29% in community-dwelling populations and 14-33 % in long-term care populations. In the only acute-care hospital examined in the study, the prevalence was 10% (17).

Muscle consists of numerous muscle fibers in addition to connective tissue, blood vessels, and nerves. The interior of muscle fibers consists of myofibrils, which in turn are made up of the proteins actin and myosin. Actin and myosin are essential in muscle contraction. In addition, in a section of myosin there is a site for binding ATP. Because of the large energy requirements involved in muscle contraction, muscle fibers have a rich supply of mitochondria generating ATP. The action

potential in muscle is triggered by a signal from the nerves in motor nerve fibers in the muscles. One motor neuron is connected to many muscle fibers, while each muscle fiber is connected to only one motor neuron. A motor unit is defined as one motor neuron with its connecting muscle fibers. Based on the rate of the muscle contraction, muscle fibers are divided into two categories: type I (slow-twitch) and type II (fast- twitch). Another division between the muscle fibers is based on their capacity for anaerobic or aerobic production of ATP: fibers that make ATP by oxidative phosphorylation are said to be oxidative, and fibers that are able to cover their energy needs by glycolysis are said to be glycolytic. The type-I fibers are slow-twitch, oxidative fibers, while the type-II fibers are divided into two subcategories: fast-twitch oxidative fibers (type IIa) and fast-twitch glycolytic fibers (IIb). After birth, the formation of new muscle cells is limited, but with growth the size of the muscle fibers increases, and therefore so does the muscle volume. Satellite cells are precursors of skeletal muscle cells that are found in mature muscle cells. Hence, there are possibilities for forming new muscle fibers. Satellite cells can also repair existing cells (18).

The processes that lead to sarcopenia with aging are complex (**Figure 1**). With aging the muscle mass is reduced. This is due mainly to loss of fibers. It has been suggested that about 60 % of the lost fibers are the type-II fibers (19). Type-II fibers are the most important muscle fibers for preventing falls, due to the "fast" response that is necessary to prevent falling. Loss of the type-II fibers is one reason for the increased risk of falling in old age (20). Other changes include a reduction in the amount of satellite cells, which are important for muscle growth and repair (21). The composition of the muscle changes. In addition, the muscle fibers are infiltrated with fat and connective tissue (*myosteatosis*) (1), and there are fewer motor units in advanced age (22). Owing to the age-related increase in mutations in mtDNA, there is also a reduced synthesis of mitochondrial proteins and/or an increased production of abnormal proteins. Both these processes are associated with reduced oxidative capacity, and hence with slower recovery after exercise and reduced mitochondrial function (23).
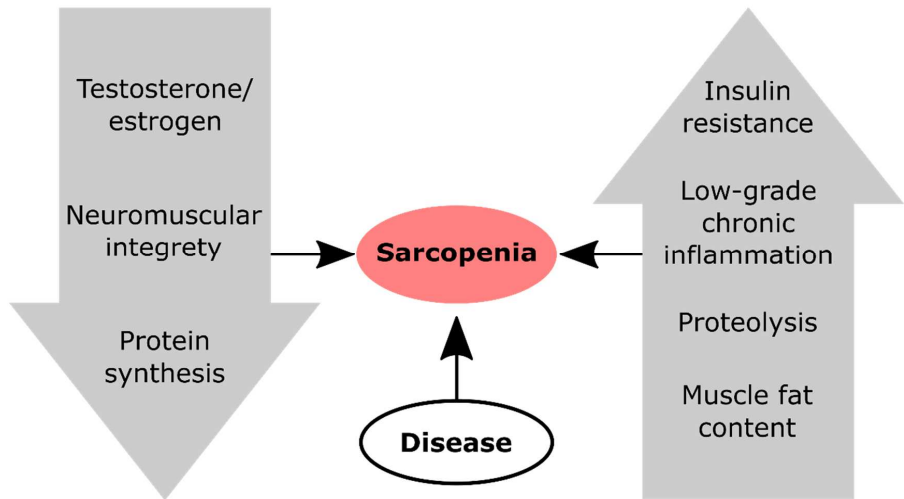
Figure 1: Overview of the processes leading to sarcopenia. The (primary) age-related changes that occur include reduced levels of sex hormones, reduced neuromuscular integrity, reduced protein synthesis, and presence of low-grade chronic inflammation. All this is accompanied by increasing insulin resistance, increased muscle fat content and increased proteolysis. In addition, presence of acute or chronic disease can affect the development of sarcopenia (secondary).

Several endocrine changes occur as a person ages, including a decrease in the levels of sex hormones (i.e. testosterone and estrogen), insulin, corticosteroids, and growth hormone/insulin-like growth factor 1. This may influence the protein metabolism in both the anabolic and the catabolic state (24). "Anabolic resistance" is a term used for the phenomenon in which the response of protein synthesis to intake of dietary protein is diminished (25). Other changes that occur include increased proteolysis, insulin resistance, and low-grade chronic inflammation. The development of sarcopenia is to some degree physiological, and only becomes pathological when it exceeds a certain degree. The risk factors that contribute to the development of sarcopenia include age, gender, low physical activity (26), inadequate nutritional intake (24), smoking (27)), and presence of chronic diseases (13); these factors often occur in combination.

The serious consequences of sarcopenia are increased frailty including reduced mobility, increased morbidity, and mortality. The functional decline and disability in older persons is strongly correlated with sarcopenia (9, 11) these individuals are consequently at a higher risk of falling and hence suffering an injury or fracture (27). Other consequences include increased susceptibility to hospital infections (28).

1.1.3 Bone mineral density and osteoporosis

Osteoporosis, derived from the Greek *osteon* = bone and *porosis* = porosity, is a systemic skeletal disorder characterized by porous bones, loss of bone mass, and deterioration of the bone structure. It is a condition without any clinical signs or symptoms until a low-energy fracture (fall from standing height or less) makes its presence evident. Thus, osteoporosis may be diagnosed in the presence of a low-energy fracture or as defined by the WHO (29, 30): it is present when the bone mineral density (BMD) is 2.5 standard deviations below that of a young, healthy adult, applying age and gender-specific cutoffs. BMD is measured predominantly by DXA, which has become the gold standard for BMD measurements (31). The BMD at the femoral neck is regarded as the best measure of osteoporosis and a predictor of the risk of low-energy fracture (32). The factors that contribute to the development of low BMD are the unmodifiable ones - age, female sex, ethnicity, and early menopause, but also some modifiable ones: smoking, high alcohol consumption, physical inactivity, weight loss, and low body mass index (BMI). Factors that are thought to be protective are e.g. use of estrogen therapy, physical activity, and some dietary constituents that will be outlined in detail later on (33).

Owing to the scarcity of clinical symptoms before a fracture occurs the prevalence of osteoporosis is uncertain, but it is estimated that 200 million people worldwide suffer from this condition (34). Hip fractures are often used as an index of osteoporosis prevalence. There are geographic variations in the incidence (35), even within Europe (36). Since life expectancy differs between different countries, this

also affects the prevalence of osteoporosis because loss of bone mass becomes more pronounced with advancing age.

Bone tissue is of two types, cortical and trabecular. Cortical bone has a structural function and forms the outer, hard layer of most bones. Trabecular bone has a metabolic function, comprising bone marrow production of red blood cells and bone cell precursors. Trabecular bone forms the interior of most bones, and consists of a sponge-like web of thin calcified trabeculae (37). Trabecular bone undergoes remodeling seven times faster than cortical bone, and hence the sites of trabecular bone, like wrists, the spine, and the hips are more susceptible to fractures. After reaching the peak bone mass in the third decade of life, the bone remodeling phase sets in (33), a constant cycle of bone resorption (by osteoclasts) and bone formation (by osteoblasts). Osteoblast activity is stimulated by 1,25-dihydroxyvitamin D (1,25-$(OH)_2$D) (38). With increasing age, osteoclast activity tends to exceed the rate of re-mineralization, and therefore the bone mineral content and density begin to decline. Other factors that contribute to decreased bone mineralization are decreased vitamin D synthesis in the skin, decreased absorption of calcium, and low-grade systemic inflammation (39). One of the systemic factors that controls the bone modeling cycle is estrogen. Examination of cortical bones has demonstrated that bone loss is closely linked to estrogen deficiency. On the other hand, the loss of trabecular bone seems to be independent of estrogen depletion (40). Several mechanisms may be involved in the menopausal loss of bone mass. Reduced estrogen production increases the production of pro-inflammatory cytokines. Binding of the receptor for nuclear factor κβ ligand (RANKL) to receptor activator of nuclear factor κβ (RANK) is essential for osteoclast activity and differentiation, but the activity of RANKL increases postmenopausally, leading to an increased activity of osteoclasts without a corresponding increase in the activity of osteoblasts (41). This contributes to a net loss of bone mass. The menopause in women and the associated loss of endogenous estrogen has historically been proposed as the sole reason for the accelerated loss of BMD with age (42), but later this has been questioned (43). Estrogen therapy was at one time used as replacement during menopause, but its use is now less common owing to its suspected side effects. However, the positive effects on osteoporosis and

hip fracture have been emphasized (44). Bone resorption is also inhibited by testosterone. The testosterone levels in men tend to decrease with age, and men with testosterone deficiency are more often osteoporotic and have a more rapid bone loss compared with men having normal testosterone levels (45). However, this effect on the BMD seems not to be as dramatic as estrogen deficiency in women.

Osteoporosis increases the risk of hip, wrist, or spine fractures, which in turn may lead to loss of physical function and independence in activities of daily life, to poor quality of life, and to increased mortality. In people who have already suffered a low-energy fracture, the risk of having a new fracture at any site is considerably increased (46, 47). The consequences of hip fractures will be described in details in the following section.

1.1.4 Hip fractures

The occurrence of a hip fracture is strongly correlated with low bone mineral density (48). However, injury due to low energy fall or trauma is a prerequisite for a fracture. The low BMD increases risk of a fracture caused by the fall. Established modifiable risk factors for hip fracture include low BMI, recent weight loss, low protein intake, low calcium intake, sarcopenia, vitamin D deficiency and smoking (49-52). Fractures of the hip can occur in several locations (**Figure 2**). Further outlining of fracture sites and surgical procedures is beyond the scope of this thesis.

Worldwide, about 1.7 million people suffer from a hip fracture annually (34). Norway has the highest prevalence of hip fractures in the world, about 8,400 people having been hospitalized for this condition in 2015 (53). The mean age of the hip fracture patients in Norway in 2015 was 80 years, and about 70% of these patients are women. Within Norway itself there are large regional differences in the incidence of hip fractures (54). In many countries, including Norway, the population rates of hip fractures have declined over the last decade (55).
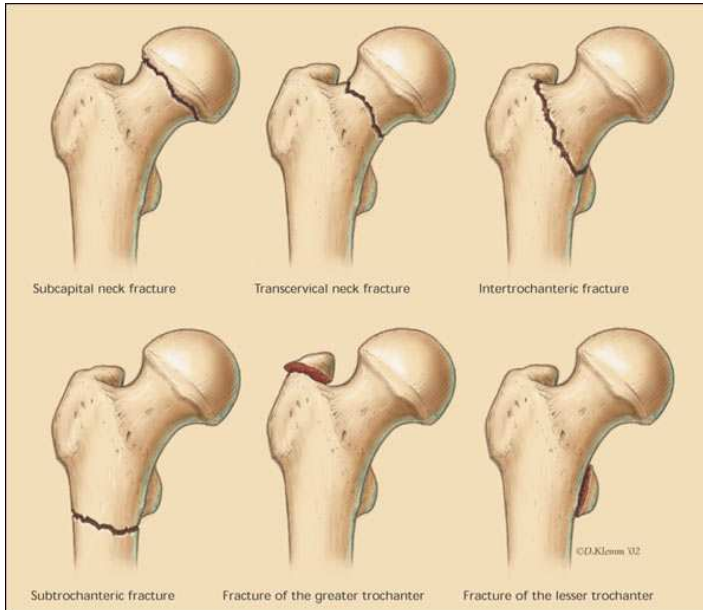
Figure 2: Overview of the various types of hip fractures. Hip fractures are divided into two main categories: intracapsular (femoral neck) and extracapsular (trochanteric and subtrochanteric). Source: https://www.physio-pedia.com/File:Figure1_hip_fracture.jpeg

This work has been released into the public domain by its author.

Hip fractures present major problems both on the individual level and for the society. Hip fracture patients have impaired physical function and an increased risk of mortality, and they require more health services. Hip fractures are thus associated with increased health care expenditure (56), comprising the costs of hospitalization, surgery, rehabilitation in specialized nursing home facilities, and the need for long-term home care or nursing home residency for those with reduced mobility and abilities in their daily life (57). It has been estimated that about half of the patients who had been community-dwelling prior to the fracture required long-term care in nursing homes or at home after the fracture (48). These patients are also at an increased risk of a second hip fracture. In a study from Norway (47), the estimated 10-year risk of a second hip fracture was 15% in women and 10% in men. The mortality rates after hip fracture remain high (20-35%), and are positively associated

with pre- and postoperative delirium, dementia, anemia, catheter-related urinary tract infections, and pneumonia (58-60), residence in a nursing home, and the male sex (61, 62).

Hip fracture has a major impact on physical functioning of the patient. Early recovery of mobility is decisive for the long-term prognosis (63, 64). It is estimated that about half of the patients who had been able to walk independently before the hip fracture required an aid or became non-ambulatory within one year after the fracture (63-65). Low muscle mass and low strength predict an impairment of mobility one year after the fracture (66). These results suggest that presence of sarcopenia has huge implications for recovery of physical function and independence after a fracture.

1.2 Vitamin D

1.2.1 Dietary intake

Vitamin D, or calciferol, comes in two different forms, ergocalciferol (D_2) and cholecalciferol ($D_{3,}$), which differ only in their side-chain structure (67). The main dietary source is cholecalciferol, as there are only a few dietary sources of ergocalciferol: yeast and mushrooms (68). The main dietary source of cholecalciferol is fish, primarily fatty fish. Other sources are eggs, liver, cod liver oil, and fortified foods (69). In Norway, fish and fish products account for about 40% and eggs for about 17 % of the vitamin D intake, while 34 % comes from fortified skimmed milk and margarine. Norwegians in general do not have an adequate intake of vitamin D, but taking into account the contribution from cod liver oil, the average intake is close to the recommended value (70).

1.2.2 Skin synthesis

Dermal synthesis is the major natural source of vitamin D (71). Previtamin D_3 is synthesized non-enzymatically in skin from 7-dehydrocholesterol during exposure to ultraviolet B (UVB) light at wavelengths of 290-315 nm. Previtamin D_3 undergoes a temperature-dependent rearrangement to form vitamin D_3 (72). The season and the latitude (solar zenith angle) determine the availability of UVB light of the correct wavelength (73). In northern cities like Bergen (60° 23' 34.76" N), the sunlight available for skin synthesis is only present approximately from April to September (74). This means that during the fall and in wintertime Norwegians have to rely on vitamin D reserves built up during the summer or must ensure adequate vitamin D intake. The skin synthesis of vitamin D is affected by several factors. Besides the skin type and the use of sun blockers, aging has a major influence, since the synthesis process is reduced by the smaller availability of 7-dehydrocholesterol (75).

1.2.3 Functions

In addition to the long-known parts played by vitamin D in calcium and phosphate metabolism, several functions of vitamin D in the body have recently been discovered. Because of the scope of this thesis, only functions related to bone metabolism and muscle strength are discussed further on.

The main function of vitamin D is maintaining calcium and phosphate homeostasis. The calcium plasma levels are maintained within a narrow range (76). In its hormonal form 1,25-$(OH)_2$D, vitamin D maintains calcium and phosphate homeostasis by acting predominantly on three different organs: the intestine, kidneys, and bone (77). In the intestinal enterocytes, 1,25-$(OH)_2$D engages in the active transport of calcium by binding to vitamin D receptors, which leads to the synthesis of calcium-binding proteins. This in turn regulates the transport of calcium across the cell membrane. Regulation of the renal production of 1,25-$(OH)_2$D is mediated via receptors in the parathyroid gland, which sense if the calcium levels are low and therefore release parathyroid hormones (PTH) (78). The increased production of 1,25-$(OH)_2$D leads not only to increased absorption of calcium in the intestine, but also to resorption of calcium in the kidneys and bone (79). An increase in the calcium levels inhibits PTH-secretion, and cells in the thyroid gland begin to secrete calcitonin. Calcitonin both blocks the resorption of calcium from bone and suppresses the production of PTH. The calcium and phosphate homeostasis is closely linked together. An increase in phosphate levels stimulates secretion of PTH and suppresses formation of calcitonin (67). The increased PTH levels increase renal production of 1,25-$(OH)_2$D, which stimulates osteoblasts and osteocytes to produce fibroblast growth factor 23 (FGF23). The increased FGF23 and PTH levels reduce reabsorption of phosphate in the kidneys and thus lower the phosphate levels. Action of FGF23 in the proximal tubuli is dependent on a transmembrane protein known as Klotho. FGF23 creates a multiloop feedback system by decreasing PTH secretion and down-regulation of 1α-hydroxylase and thus the 1,25-$(OH)_2$D levels (80).

Vitamin D has many known functions other than maintaining calcium homeostasis. Muscle weakness in rickets was described by Glisson already in the 17th century (81). Muscle weakness is still considered a symptom in vitamin D deficiency (20, 82) and in older persons (83, 84). In patients with osteomalacia, myopathy has been observed as one of the symptoms, which is reversible by treatment with vitamin D (85). However, it is uncertain whether a mild deficiency or insufficiency of vitamin D has a significant effect on muscle strength. The role of vitamin D in muscle function is complex and not fully understood, but some of the possible mechanisms are outlined below.

The non-genomic effect of vitamin D is the most investigated muscle property. Muscle contraction is dependent on $1,25\text{-}(OH)_2D$-stimulated phosphorylation of proteins which regulates CaATPase, a transport protein that transfers calcium ions into the sarcoplasmic reticulum. In addition, the genomic effects of vitamin D are exerted via the expression of the vitamin D receptor (VDR), a nuclear receptor with a high affinity to $1,25\text{-}(OH)_2D$ which regulates protein synthesis by its action (86-88). There are several VDR genotypes, which appear to give rise to differences in muscle strength (89). There is also a decline in the expression of VDR with increasing age, which may play a role in the age-dependent muscle weakness (90).

1.2.4 Vitamin D status

The serum levels of 25-hydroxy vitamin D (25-(OH)D) are used as the proxy for the body's vitamin D stores because of its stability and long half-life (67). The methods used for the determination of 25-(OH)D levels have traditionally been immunoassays, but are now increasingly replaced by LC-MS/MS methods, which are more specific and easier to standardize. There is no agreement on the optimal level of 25-(OH)D, but the Institute of Medicine (IOM) and other bodies have accepted > 50 nmol (67, 91). However, the Endocrine Society (92) refers to an optimized level of > 75 nmol/L. An overview is presented in **Table 3.**

Table 3: Overview of the serum 25-(OH)D level cutoffs used for classification of the vitamin D status by the Institute of Medicine (IOM) (67), Nordic Nutritional Recommendations (NNR5) (91) and The Endocrine Society (92).

Vitamin D status	IOM		NNR5		Endocrine Society	
	nmol/L	ng/ml	nmol/L	ng/ml	nmol/L	ng/ml
Deficiency	< 30	< 12	< 25	< 10	< 50	< 20
Insufficiency	30 - < 50	12 - < 20	30 - < 50	12 - < 20	53-73	21-29
Sufficiency	≥ 50	≥ 20	≥ 50	≥ 20	≥ 75	≥ 30

Deficiency of 25-(OH)D is widespread in Europe, and the seasonal variation of the serum 25-(OH)D levels, with lower levels in winter, contributes to the problem (93). During the months without any dermal synthesis, people have to rely on dietary intake. A recent study (94) has investigated the prevalence using standardized 25-(OH)D values from national surveys in Europe. The conclusion was that 25-(OH)D deficiency (< 30 nmol/L) is common, occurring in about 13% of children and adults in Europe, with insufficiency (25-(OH)D levels < 50 nmol/L), running at about 40%.

1.2.5 Dietary recommendations

In line with scientific developments and the application of the risk assessment framework, the dietary recommendations in many countries have been modified (95, 96).). In 2011, the IOM proposed new Recommended Daily Allowance (RDA) (67). In this report, the RDA was 600 IU/day, which was assumed to ensure serum 25-(OH)D levels higher than 50 nmol/L for 97.5% of the healthy population in the 1-70 years age group. In line with the IOM report, in 2014 the RDA in the Nordic countries was raised from 200 to 400 IU (91). When the diet and the supplements are considered together, the Norwegian intake of vitamin D from dietary sources (mainly from fish and cod liver oil) is among the highest in Europe (97). The dietary

recommendations for vitamin D will probably change again as the gaps in our knowledge are filled with new insights. The present recommendations for the US, Europe, and the Nordic countries are listed in **Table 4**. The dietary recommendations are based solely on dietary intakes of vitamin D needed to achieve sufficient levels without any contribution from skin synthesis.

Table 4: Recommended intake of vitamin D (µg) in the US (98), European Union (EFSA) (99), German-speaking countries (100) and the Nordic countries (Norway, Sweden, Denmark, Finland and Iceland) (91).

Age groups (years)*	US (µg)	European Union (µg)	German-speaking countries (µg)	Nordic dietary recommendations, 2012 (µg)
Infants	10	10	10	10
Children, adults and older persons (> 1 year)	15 >70 years: 20	15	20 >65 years: 20	10 ≥ 75 years: 20

1 µg = 40 IU

* The age groups are not always the same

1.3 Fish consumption

1.3.1 Nutrient content

Fish in general is an excellent source of protein, n3 polyunsaturated fatty acids (n3 PUFAs), vitamins, and minerals including iodine and selenium. However, the nutrient content of fish varies between species. A rough classification according to the overall fat content is useful for identifying fish with high vitamin D contents, although other classifications are also in use. Lean fish contains on average less than 2 g fat per 100 g of fish fillet, and fatty fish contains more than 8 g of fat. Fatty fish like herring, mackerel, and salmon are good sources of vitamin D and n3 PUFAs (101), and are the main source of vitamin D when endogenous synthesis is low. The amounts of protein in fatty and in lean fish are about the same, about 15 to 20 g of protein per 100 g fish fillet (102). Liver from lean fish (cod) is the source of cod liver oil, which is extensively used in Norway as a supplement (it provides both vitamin D and n3 PUFAs).

1.3.2 Dietary recommendations and intake

Norway is among the countries with highest fish consumption in Europe, together with Spain and Portugal (103, 104); the other European countries consume much less fish (105, 106). Fish as a food group is explicitly mentioned in food-based dietary guidelines. In Norway, the recommended intake is 2-3 fish servings per week (corresponding to 300-400 g), of which 200 g should be fatty fish (107). Other countries also recommend consuming fish at least 1-2 times a week (108). The differences between the recommended amounts can be explained by differences in the evaluation of the health benefits of fish (109-112) and the risks of environmental pollutants contained in fish (113, 114). A detailed discussion of the health benefits of fish and n3 PUFAs is beyond the scope of this thesis, but can be found in (113-116).

Norway and Spain give the highest recommendations for fish consumption, which is in line with the high intakes of fish in these countries. With its long

coastline, Norway has a long tradition of eating fish, though again there are differences within the Norwegian population (70), with older people consuming more fish than the younger, women eating less fish than men, and people living along the coast eating more fish than those living inland (117, 118). However, despite the high average fish consumption, only 20% of the men and 25% of the women are consuming as much fish as recommended (70).

1.4 Dietary predictors of muscle mass, bone mineral density and risk of hip fractures

Diet is a modifiable factor in the prevention of low muscle mass and low BMD. It is clearly necessary to distinguish between the role of diet in the primary and in secondary prevention of these conditions. In addition, although dietary factors are definitely associated with healthy aging and with the maintenance of muscle mass they cannot be considered in isolation from a healthy lifestyle that also includes physical activity, giving up smoking and weight maintenance (33, 119). However, because of the scope of the present thesis, only nutritional factors and only those related to fish consumption will be discussed here.

1.4.1 Nutritional factors in loss of muscle mass, strength and physical funtion

In addition to specific nutrients that have been investigated in relation to the maintenance of muscle mass and strength, the need for an adequate energy intake has been emphasized as the most important dietary measure (119). Adequate energy intake is required to ensure that dietary protein is utilized in muscle protein synthesis (24) rather than in gluconeogenesis, and to prevent the breakdown of muscle protein. Dietary protein is the most important nutrient concerning muscle mass and strength. However, the evaluation of protein intake in primary and secondary prevention of muscle mass and strength losses is complicated by the dependence of protein requirements on adequate energy intake, physical activity, and the health status. While the NNR5 (91) are directed at healthy older persons, and thus recommend 15-20 E% of protein intake (equivalent to about 1.1 – 1.3 g/kg body weight (BW)), other scientific societies have focused on the role of protein in disease and have recommended higher protein intakes (16, 120).

The evidence on dietary protein intake in primary prevention of muscle mass loss is derived mainly from observational cohort studies (121-125), and less from intervention studies using protein supplementation (126, 127). In the cohort studies, a

higher protein intake was associated with smaller loss of lean body mass (128), less pronounced frailty (129), higher hand-grip strength, and better physical function (121-125). Most of these studies were done on older persons below the age of 80, and one of the few studies with very old persons (age > 85 years) reported that an intake of less than 1 g protein/kg BW was negatively associated with muscle strength and performance, especially in women (130). Regarding secondary prevention, it seems that regaining muscle mass is even more difficult to achieve. For example, one well-designed intervention study did not find any increase in muscle mass in older adults after 12 weeks of intervention with 40 g protein per day (131).

Vitamin D has also attracted much interest in connection with muscle mass and function. Many older persons suffer from vitamin D deficiency (77) and this has been associated with reduced mobility, but not with muscle strength (132, 133). Low vitamin D status has also been associated with development of sarcopenia in the prospective Longitudinal Aging Study Amsterdam (134). Thus, there is evidence from observational studies that vitamin D status is associated with reduced muscle mass and mobility. Several RCTs were conducted to test whether vitamin D supplementation would improve physical function in older persons. A major outcome measure was the risk of falls, which is often used as a proxy for low muscle strength and function (135, 136), although the risk of falls are multifactorial (137). Results of these RCTs have been summarized in several meta-analyses (135, 138, 139). These were complicated by differences in the vitamin D supplementation strategies (doses, metabolites, routes of administration) and in the population studied (community-dwelling vs. institutionalized, vitamin D status at baseline). Thus, results are inconsistent and there are doubts whether vitamin D supplementation can reduce the risk of falls. Other outcomes of physical function in older persons have also been addressed in a number of RCTs, which again have been summarized in several meta-analyses (140-145). Again, it is difficult to compare studies due to difference in the population included, the vitamin D supplementation strategies and the outcomes of physical function.

1.4.2 Nutritional factors associated with bone mineral density and the risk of hip fractures

A possible role of diet in the development of osteoporosis has been discussed for more than 40 years, with numerous studies having been published on primary and secondary prevention of osteoporosis and hip fractures (33). In addition to single nutrients, the role of sufficient energy intake and of nutritional status has attracted particular attention. In primary prevention of osteoporosis, the nutrients in question include the total protein intake and the sources (146, 147), calcium (148), vitamin D (149), vitamin K (150, 151), and n3 PUFAs (152, 153). Since osteoporosis develops throughout a person's lifetime, in primary prevention the diet during childhood and adolescence is also of interest (33).

Since bones contain large amounts of protein, it seems reasonable to look at the protein intake as a beneficial nutrient in the prevention of osteoporosis and hip fracture. Historically, a high protein intake was thought to be unfavorable, as it would increase the urinary calcium excretion. However, recent studies have not found any adverse effects of a high protein diet, owing to compensatory increased intestinal calcium absorption that could most probably replace the urinary calcium losses (154). Much research has been aimed at identifying the protective effects of specific kinds of proteins (animal vs. plant, dairy and soy), but the evidence for beneficial effects of specific kinds of protein is spars (155-159). A high protein intake may have a favorable effect on BMD, especially in combination with adequate intakes of calcium and vitamin D (146, 158, 160), although this may not be translated into a reduced risk of fracture (146, 154). This absence of beneficial effects was attributed to the proportions of protein and calcium in the diet, a protective effect on the risk of hip fracture being only present with diets high in calcium and protein (161).

The effects of vitamin D supplementation on bone health and on fractures with both intermediate and clinical outcomes have been investigated in numerous RCTs, even before the current hype concerning vitamin D. The overall results suggest that de effect of vitamin D (with or without calcium) is still uncertain in preventing fractures (primary or secondary prevention), including hip fractures (162-164),

though this does not preclude a part played by vitamin D in the life-long development process of osteoporosis (33). Indeed, a low vitamin D status has been associated with low bone mineral density (165), and an increased risk of hip fractures (149).

The association between fish intake and BMD has been investigated in populations from the US, China, and Spain with mixed results (166-170). Overall, there is a trend toward a positive association between fish consumption and BMD (171). However, no association between fish consumption and BMD was found in a review of studies on dietary patterns that included fish consumption (172). As part of fish, n3 PUFAs have attracted much attention for their potentially anti-inflammatory properties and their proposed effect on bone metabolism (171, 173). However, the effect of n3 PUFA intake on the development of osteoporosis is still unclear, both from observational studies (166, 174) and from RCTs (175, 176). When clinical outcomes are considered, the association between fish intake and the risk of hip fractures is unclear (167, 177, 178).

1.4.3 Nutritional status and hip fractures

From many studies involving hip fracture patients, it is evident that these patients predominantly have a lower BMI than their peers of comparable age (179, 180). Further in-depth investigations have shown that these patients also have a low food intake and are frequently undernourished (50, 180-183). These observations may indicate that a low muscle mass already before the occurrence of the fracture is common, but few studies have been done on this point (66). Hip fracture patients have a hypermetabolic state that persists for several weeks after the fracture (184). It is therefore not surprising that weight loss is common after the fracture (185-188), although this has not been studied in any detail. Only one study was found that reported on the association between weight loss after the fracture (where weight measurements had been made) and functional outcomes (185).

The literature search for the present thesis was finalized on June 11, 2018.

2.   Rationale for the present study

With increasing life expectancy, healthy aging has become a focus of health research. Europe has experienced a period of wealth and abundant food supply for more than six decades, which has shifted the focus in nutrition research from undernutrition and nutritional deficiencies to the role of nutrition in prevention of chronic disease and maintenance of health also in old age. As older persons are a heterogeneous population group, we chose, in order to reduce heterogeneity, to focus on community-dwelling older persons with normal[1] cognitive status. We were specifically interested in the effect of vitamin D supplementation on muscle strength and mobility, and the association between fish intake, osteoporosis and hip fractures. In addition, we were interested in the effect of weight changes on mobility after a hip fracture. Different approaches were applied to study these, including a meta-analysis and a clinical study, as well as cross-sectional and longitudinal analyses of a population-based cohort.

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[1] Cognitive status was not formally tested. However, when ability to sign informed consent is taken as a proxy for normal cognitive status, it can be stated that all participants in the HUSK- cohort provided informed consent at inclusion, as well as the patients admitted for a hip fracture that participated in the clinical study. The meta-analysis was based on RCTs that also require informed consent.

3. Study objectives

Main objective

The overall aim of the PhD-project was to investigate nutritional factors, with special attention to fish intake and vitamin D on age related changes in muscle and bone mass. The aim was to summerize the effect of vitamin D supplementation on muscle mass and strength. Further, it was the aim to investigate the association of fish intake with bone mineral density and the risk of hip fractures. Finally, we wanted to study the effect of weight changes on mobility with a follow up of two months after a hip fracture. The PhD-project consists of three parts (meta-analysis, data from a cohort and a clinical trial).

Specific objectives

Paper I:

To summarize the evidence whether vitamin D supplementation with or without additional calcium supplementation improved physical function and muscle strength in community-dwelling older persons (systematic review and meta-analysis of randomized clinical trials).

Paper II:

To investigate the association between total fish intake, lean and fatty fish intake and bone mineral density in the HUSK in a cross sectional analysis.

Paper III:

To investigated the association between total fish intake and the risk of future hip fracture in HUSK-participants born in 1925-27.

Paper IV:

To investigate the effect of energy intake and weight change on mobility and physical function in Norwegian hip fracture patients up to three months post fracture.

4. Subjects and methods

4.1 Study population and design

4.1.1 Paper I: Systematic review and meta-analysis

Relevant studies were identified by a systematic search of current literature using PubMed, Embase, Medline, Web of Science and the Cochrane Library, followed by a manual search of the extracted articles and existing reviews. The clinical trial registry 'ClinicalTrials.gov' was also searched for unpublished trials. The search covered the period up to April 13, 2016. The search terms are found in **Appendix I** (**Supplementary material)**. The inclusion criteria are stated in **Table 5**. Only the outcomes comprising hand grip strength (HGS) and timed-up-and-go (TUG) were investigated in a sufficient number of studies to perform a quantitative meta-analysis, whereas other outcomes of muscle strength and mobility were only included in the systematic review.

Table 5: The inclusion criteria for the systematic review and meta-analysis.

Design	Randomized controlled trial
Participants	Older persons > 65 years of age Humans Community-dwelling
Intervention	Vitamin D supplementation – all forms and all doses, with or without calcium supplements or dietary advice
Comparator	Low dose of vitamin D or vitamin D metabolites or placebo, with or without calcium supplement
Outcome measures	
Systematic review	Measures of muscle strength and mobility
Meta-analysis	Hand grip strength (HGS) Timed-up-and-go test (TUG)

Briefly, HGS is a method for measuring muscle strength. HGS was measured with a hand-held dynamometer (189-191). The average of 2-3 measurements are calculated (191). TUG is a method for testing the mobility of a person. Subjects are observed and timed from the instant they rise from an armchair (seat height 48 cm, arm height 68 cm), walk 3 or 4 meters, navigate an obstacle on the floor, and return to a fully seated position in the chair. Each subject perform the test three times and the fastest of the three trials is finally recorded (192). A description of the other physical performance tests used in the studies that are included in the systematic review is described in **Appendix I (Supplementary material**)).

4.1.2 Papers II and III: The Hordaland Health Study (HUSK)

The Hordaland Health Study '97-'99 (HUSK) was conducted during 1997-1999 as a collaboration between the National Health Screening Service, the University of Bergen and local health services (https://husk-en.w.uib.no/). The main purposes of HUSK were to quantify the burden of potentially modifiable risk factors in the general population and to lay the ground for etiological studies of specific risk factors/conditions in relation to diseases of interest. In HUSK, all individuals living in Hordaland county and born during 1953-1957 (29,400) were invited. In addition, 2,291 men and 2,558 women born during 1950-1951, and 1,868 men and 2,470 women born in 1925-1927, who had previously participated in the Hordaland Homocysteine Study (193) were invited. Both the last two birth cohorts were used for the analysis in Paper II. In Paper III, only the older cohort was suitable for analysis due to the low number of hip fractures among the middle-aged participants.

Dietary intake

The participants were given a 169-item semi-quantitative food frequency questionnaire (FFQ) at the day of the health examination that was filled out at home and returned by mail to the HUSK Project Center in Bergen, Norway (**Appendix V**). The FFQ was analyzed using the database "Kostberegningssystem" (Kostberegningssystem, version 3.2; University of Oslo, Norway).

Bone mineral density

The participants received an invitation to measure their BMD. About 5,377 participants underwent a DXA measurement (One stationary, fan beam dual X-ray densitometer; Expert-XL, Lunar; Madison WI, software version 1.72 and 1.90). The left hip was scanned unless there was a history of surgery or fracture, and the procedure followed the manufacturers guide. DXA measurements were performed between May 11, 1998 and August 8, 2000 (194). These data were used in Paper II in a cross-sectional design. A comparison between the reference values of the manufacturer (Lunar) of the DXA and the HUSK population was performed and the reference values was found suitable in a clinical setting for use in the Norwegian population (195).

Hip fracture

The number of hip fractures that occurred between 1997-1999 and December 31, 2009 were registered by electronically searching the records from the six hospitals serving Hordaland County for diagnosis codes that coded for hip fractures. The extraction process has previously been described (196, 197). Information on mortality was obtained from the Norwegian National Registry. The hip fracture data were used in Paper III.

Other measurements

All participants took part in a one-day visit at the HUSK Project Center where several health examinations were performed including blood sampling, height and weight measurements. More information can be found at http://husk-en.b.uib.no/

4.1.3 Paper IV: Weight change and mobility after hip fracture

Paper IV was a longitudinal observational study on hip fracture patients with a follow-up of three months. The patients were recruited from both Haukeland University Hospital and Haraldsplass Deacon Hospital (both located in Bergen) from February of 2014 until December of 2015. Anthropometric and functional measurements and 24-hour recall (24HR) were conducted at three visits for every patient, Visit 1 at the hospital (immediately after surgery), at Visit 2 at rehabilitation (2-3 weeks after discharge), and Visit 3 at home (2-3 months after the fracture). The inclusion criteria were age above 60 years, hospitalized for a first hip fracture, able to walk without aid, community-dwelling before the fracture and normal cognitive function (the cognitive function was not tested in the study, but was considered by a nurse at the hospital or at the rehabilitation unit). Trained clinical dieticians or nutritionists performed all anthropometric and bioelectrical impedance analysis (BIA).

24-hour recall (24HR)

To assess the food consumption of patients at all visits, 24HR was conducted, following the USDA interview guideline (198). Portion size was estimated using a booklet with four different portions shown or was estimated in household measurements or number of items consumed. Data was entered in the online dietary tool "Kostholdsplanleggeren' (www.kostholdsplanleggeren.no) which is based on the official Norwegian food composition table and edited by the Norwegian Food Safety authority and the Norwegian Directorate of Health (www.matvaretabellen.no).

Bioelectrical impedance analysis (BIA)

BIA is a tool for calculating fat-free mass and fat mass in a person by sending a weak electrical current through the body (199). BIA is inexpensive and can easily be used bedside in the clinic. However, BIA is sensitive to changes in electrolytes and body fluid. The method has been validated for healthy older men and women (200) and for hip fracture patients (201). Different formulas are used for the calculation of fat-free mass and appendicular lean mass (ALM) (the muscle mass at the legs and arms). In this study, the formula for calculation of skeletal muscle index (SMI) using the ALM by Kyle et al. (202) was applied:

ALM (Kyle) = -4.2111 + (0.267 x height^2)/resistance + 0.0095 x weight + 1.909 x sex -0.012 x age + 0.058 x reactance

SMI= ALM/height^2

New mobility score (NMS)

The New mobility score (NMS) was used to investigate mobility. The NMS is a self-reported, composite score of the patient's ability to get about the house, get out of the house, and go shopping alone, with an aid or not at all (**Table 6**). A cane, crutches, or a walker was regarded as an aid (203, 204). The NMS has been validated and are found to predict outcome in mobility to a similar degree as Barthel-20 and Barthel-100 (205).

Table 6: The New mobility score (NMS) from Parker et al. (203) with scores from 0-9.

Mobility	No difficulty	With an aid	With help from another person	Not at all
Able to get about the house	3	2	1	0
Able to get out of the house	3	2	1	0
Able to go shopping	3	2	1	0

Information from patient records

Blood values for albumin, haemoglobin, 25-(OH)D, CRP and creatinine were collected from DIPS (Distributed Information and Patient System for hospitals), mainly measured one day prior to surgery. Remaining variables, such as length of stay at the hospital, type of surgery and other personal information such as presence of osteoporosis, disease history (including co-morbidities), medication, blood pressure, type of hip fracture, type of fall and smoking status was also collected from the patients' records.

4.2 Statistical analyses

4.2.1 Paper I: The systematic review and meta-analysis

Other outcomes than TUG and HGS were reviewed narratively because of the low number of studies evaluating these outcomes. Only TUG and HGS were present in more than three studies and thereby suitable for a quantitative meta-analysis. We used RevMan, version 5.3 (Cochrane collaboration) (206) for the analysis, with the outcome being represented by Forrest plots. Weighted mean differences for vitamin D versus placebo/control were calculated by subtracting the mean of the outcome of interest at the end of the study from the mean at baseline. Standard deviations (SDs) of the differences were calculated using a formula provided by the Cochrane Handbook (207), applying correlation coefficients of 1.0 for the HGS and 0.8 for the TUG-test. Because significant heterogeneity was observed between studies with a fixed effect model, we finally applied a random effects model. Studies that included more than one intervention group (208) were treated by dividing the number of subjects in the control group by the number of comparisons at the same time as retaining the mean (SD) of the change according to the Cochrane Handbook (207). Subgroup analyses were conducted with predefined study characteristics: baseline vitamin D status, oral administration of the supplement, daily dose of vitamin D, placebo group, supplementation with vitamin D_2 or D_3, and advice on calcium supplementation to explore possible reasons for the observed heterogeneity (209, 210).

4.2.2 Papers II and III: The Hordaland Health Study (HUSK)

In both Papers II and III, continuous variables were presented as means and SD, and categorical variables as percentages. Differences between sex and age were assessed using Mann-Whitney U test for continuous variables and Fischer's exact test for categorical variables. In Papers II and III, fish intake was categorized into quartiles, calculated separately for each sex and age group. Differences in characteristics across

quartiles of total fish intake were analyzed using linear regression for continuous variables and logistic regression for dichotomous variables.

In Paper II, the association of fish intake with BMD was analyzed both in sex-specific quartiles of fish intake and fish intake as continuous variable. Multiple linear regression analyses were performed to assess the association between intake of either total, fatty or lean fish and BMD (by age group and sex) with adjustment for potential confounders. Due to missing values in confounders (1.3-6.2%), the multivariable analysis included 4,279 participants whereas the energy-adjusted model included 4,656 participants. There were no non-consumer of total fish. The number of non-consumers of lean and fatty fish was low, 405 (8.7 %) and 255 (5.5 %) respectively. The non-consumers were categorized in quartile one.

In Paper III, fish intake was introduced in the analyses a) in four categories (based on sex-specific quartiles) and b) as a dichotomous variable (comparing the lower quartile, Q1, to the three upper quartiles (Q2 to Q4). To explore visually the association between fish intake and risk of hip fracture we used Cox proportional hazards regression model fitting fish intake using restricted cubic splines with three knots. Cox proportional hazards regression models with death as competing risk were used to estimate the risk of hip fractures across fish intake quartiles. The results are presented as hazard ratios (HRs) and 95% confidence interval (CI). Models were adjusted for sex, BMI, plasma concentration of cotinine ≥85 nmol/L (a marker for recent nicotine exposure (211)), physical activity and energy intake. Further adjustment for intake of vegetables, fruit, dairy or meat, vitamin D and calcium intake or estrogen therapy (in women) did not materially change the association. In addition to overall analyses, we also conducted sex-specific analyses.

Statistical software SPSS for Windows version 25 (IBM, NY, USA) and Stata Statistical Software (Release 15.0, College Station, Texas, USA: Stata Corp LLC) were used and a two-sided p-value <0.05 was considered statistically significant.

4.2.3 Paper IV: Weight change and mobility after hip fracture

At baseline, characteristics of men and women were compared using Mann Whitney U test and Chi squared test. We also compared age and sex distribution among the two participating hospitals and weight according to whether it was self-reported or measured. Weight, dietary intake and other study outcomes were compared between Visits 1 and 2 by the Wilcoxon rank sum test for related samples. The same tests were used to compare Visits 1 and 3. The NMS at Visit 3 was used in a logistic regression analysis as dichotomous outcome variable (NMS > 5 reflecting sufficient mobility, NMS $\leq$ 5 reflecting impaired mobility). Age was used as explaining variable in all models, and only one additional explaining variable was used concurrently. Statistical software SPSS for Windows version 25 (IBM, NY, USA) was used for all calculations, and a p-value below 0.05 was regarded as significant.

4.3 Ethics

Paper I: The systematic review and meta-analysis

All studies included in the systematic review and meta-analysis were performed according to local ethical standards and the declaration of Helsinki.

Papers II and III: The Hordaland Health Study (HUSK)

The HUSK study was performed in accordance with the Declaration of Helsinki. All participants provided written informed consent. The Data Inspectorate and the Regional Committee for Medical Research Ethics (REC number: 2009/825) approved the study protocol. HUSK is registered in ClinicalTrials.gov (Clinical trial number: NCT03013725).

Paper IV: Weight change and mobility after hip fracture

The study was performed according to the Declaration of Helsinki. All participants provided written informed consent. The Regional Committee for Medical Research Ethics (REC number: 2013/2004) approved the study protocol.

5. Results

5.1 Paper 1: Systematic review and meta-analysis

5.1.1 Main results

Out of 2,408 publications obtained by systematic literature search in available databases, 15 articles were suitable for inclusion in the systematic review by our predefined inclusion criteria (see **Table 5**). From the systematic review, the majority of studies observed no improvement in muscle strength or mobility after administration of vitamin D with or without calcium supplements in community-dwelling older persons with age above 65. There were many different methods for measuring muscle strength and physical function in these publications. Only the outcomes HGS and TUG test were present in a sufficient number of articles to perform a meta-analysis. Thus, for the outcome HGS, seven studies were available, and for the outcome TUG five studies were available. In the meta-analysis, we observed a non-significant change in HGS [0.2 kg (95% confidence interval = -0.3 to 0.7 kg)] and a small, significant improvement in the TUG test [0.3 s (95% confidence interval: 0.1 to 0.5 s)] after vitamin D supplementation (for clarification, see attached **Errata**). The meta-analyses showed a high degree of heterogeneity between the studies.

A new literature search using the same search terms as in Paper I was performed on June, 11 2018 to check for new publications. Two additional studies were suitable for inclusion in the systematic review (212, 213). A narrative review of the publications are available in the next section. Searching ClinicalTrial.gov revealed several studies that had completed their study period or were recruiting; meaning several more publications on the topic are forthcoming.

5.1.2 Narrative review of additional clinical studies

Monthly High-Dose Vitamin D Treatment to Prevent Functional Decline

In the study by Bischoff-Ferrari et al. (212), 200 community-dwelling, Swiss men and women (67.0 %) with a prior fall were participating. Average age was 78 years. Method for measuring 25-(OH)D was high-performance liquid chromatography–tandem mass spectrometry. The mean baseline serum concentration of 25-(OH)D was 18 to 21 ng/mL (45 to 51 nmol/L) depending on treatment group. About 58% of the participants were defined as vitamin D deficient at baseline. The study design was a double-blinded RCT. A low dose of vitamin D was used as control instead of placebo. Participants were excluded if they continued to use calcium supplementation. Calcium supplements were not provided as part of the study protocol, and dietary calcium was not assessed. The season for blood withdrawal was stated, but the latitude of the study center was not provided. BMI as a possible covariate for vitamin D status was provided at baseline. The dropout rate was provided as a flowchart. Compliance was 94 to 98 %. Both D_3 and calcifediol were used as vitamin D supplementation and provided in oral bolus doses of 24,000 IU D_3/month (control), 60,000 IU D_3/month, and 24,000 IU D_3/month plus 300 µg calcifediol/month. Overall, the quality of the study was sufficient. A CONSORT checklist is presented in **Appendix VI**.

The primary outcome of the study was the Short Physical Performance Battery. They also measured HGS, but with another device (Martin Vigorimeter) than the RCTs included in the meta-analysis in Paper I, and therefore not suitable for inclusion. The overall conclusion of the study was that none of the vitamin D supplement strategies was beneficial for the functional decline or prevention of falls in this group of older individuals.

Alfacalcidiol and muscle strength

In the study by Setiati et al. (213), 95 older Indonesian women (age ≥ 60 years) with HGS ≤ 22 kg were participants. The median age was 70 years in both the placebo and intervention group. Serum 25-(OH)D was measured at baseline and during the study period, but the laboratory method used was not reported. The mean baseline serum concentration of 25-(OH)D was 37.5 ng/mL (94 nmol/L) in the placebo and 42.0 ng/mL (105 nmol/L) in the intervention group. The study design was a double-blinded RCT. The participants in both groups were provided 500 mg calcium per day and were not allowed to consume additional vitamin D or calcium supplementation. The season for blood withdrawal was not stated and neither was the latitude of the study center. BMI as a possible covariate for vitamin D status was provided at baseline. The dropout rate was provided as a flowchart; one participant from the treatment group and six participants from the placebo group withdrew from the study. The method for minimizing lack of compliance was addressed, but the actual compliance was not reported. A vitamin D metabolite, 0.5 µg alfacalcidiol/day, was the intervention chosen. The supplements were provided in sealed plastic packages for one month at the time. The duration of the study was 90 days. Overall, the quality of the study was sufficient. CONSORT checklist for the study is presented in **Appendix VII**.

The primary outcome of the study was HGS measured by handgrip dynamometer and 3 m TUG test. Both the outcomes would therefore be suitable for inclusion in the meta-analysis performed in Paper I. The overall conclusion of the study was that a daily supplement of 0.5 µg alfacalcidiol and 500 mg calcium significantly increased muscle strength and mobility.

5.2 Paper II: Fish consumption and bone mineral density

5.2.1 Main results

Of the total HUSK cohort, 4,656 participants were examined by DXA-measurements and answered an FFQ. The mean (SD) total fish intake was 33 (18) g/1000 kcal and was primarily lean fish. Older women had significantly lower BMD than older men and middle-aged men and women. In older women, total and lean fish intake (per 50 g/1000kcal increase) was significantly and positively associated with BMD, even after multivariable adjustments (β-coefficient 0.018, p = 0.017 for total fish intake and 0.026, p = 0.02 for lean fish intake).

5.3 Paper III: Fish consumption and hip fracture risk

5.3.1 Main results

Out of 3,327 eligible participants (age 71-74 years), information regarding food intake and hip fractures were available for 2,865 (86%). Mean (SD) follow-up time was 9.6 years (2.7). The number of hip fractures was 226 (7.9 %), 72 (5.5 %) in men and 154 (9.9%) in women. Mean (SD) total fish intake was 48 (25) g/1000 kcal in the entire cohort, with higher consumption in men (51 (25) g /1000 kcal) than in women (45 (25) g/1000 kcal). Both in men and women, lean fish was the main fish type consumed, followed by fatty fish and processed fish. The association between fish intake and risk of hip fracture was not linear and displayed a threshold, with low intake of fish being associated with increased risk of hip fracture in men (HR=1.84, 95% CI 1.10, 3.08).

5.3.2 Additional results

We performed competing risk analysis for fish intake as a continuous variable, in sex-specific quartiles, and for the lowest versus the upper three quartiles of fish intake. Death was the competing event. The results are presented in **Tables 7-9**. The results were not substantially different from the Cox proportional hazards regression presented in Paper III, and therefore not provided as tables in the paper.

Table 7: Cox proportional hazards regression with death as competing event of risk of hip fracture according to continuous baseline fish intake among the total cohort of men (n=1,1315) and women (n=1,58) in the Hordaland Health Study followed from inclusion in 1997-99 (age 71-74 years) until December 31, 2009.

	SHR	95% CI	p-value
Total fish, g/1000 kcal	1.00	(0.99, 1.00)	0.400
Lean fish, g/1000 kcal	1.00	(0.99, 1.01)	0.424
Fatty fish, g/1000 kcal	1.00	(0.99, 1.02)	0.605

Adjusted for sex, BMI (cont.), plasma cotinine above 85 nmol/L (yes/no), physical activity (none, low, moderate, high) and energy intake (cont.)

Table 8: Cox proportional hazards regression with death as competing event of risk of hip fracture according to sex-specific quartiles of baseline fish intake among the total cohort, men (n=1,1315) and women (n=1,58) in the Hordaland Health Study followed from inclusion in 1997-99 (age 71-74 years) until December 31 2009.

	Total fish, g/1000 kcal	HR	95% CI	P
Total cohort	Q2-Q4	1.00 (ref)		
	Q1	0.74	(0.54, 0.99)	0.049
Men	Q2-Q4	1.00 (ref)		
	Q1	0.56	(0.34, 0.95)	0.030
Women	Q2-Q4	1.00 (ref)		
	Q1	0.84	(0.57, 1.22)	0.360

Adjusted for sex, BMI (cont.), plasma cotinine above 85 nmol/L (yes/no), physical activity (none, low, moderate, high) and energy intake (cont.)

Table 9: Cox proportional hazards regression with death as competing event of risk of hip fracture comparing Q1 with Q2-Q4 of baseline fish intake for the total cohort (men, n=1,1315 and women, n=1,58) in the Hordaland Health Study followed from inclusion in 1997-99 (age 71-74 years) until December 31 2009.

Total fish, g/1000 kcal	SHR	95% CI	p for trend
Q1	1.00 (ref)		
Q2	0.76	(0.51, 1.12)	
Q3	0.82	(0.56, 1.19)	
Q4	0.64	(0.42, 0.96)	0.163

Adjusted for sex, BMI (cont.), plasma cotinine above 85 nmol/L (yes/no), physical activity (none, low, moderate, high) and energy intake (cont.)

5.4 Paper IV: Weight change and mobility after hip fracture

5.4.1 Main results

In the hospital, 64 patients (48 women, 16 men) agreed to participate in the study and either reported their weight (n = 30) or had their weight measured (n = 34). Half of the patients were older than 80 years. Median weight in women was 58.5 kg (IQR: 50.2, 67.8) and in men 70.0 kg (IQR: 60.8, 88.1) ($p<0.05$). Dietary intake in the hospital was assessed by 24HR after the surgery (median no of days after surgery: 3 (range 1-10) and a median energy intake of 1314 kcal/d (IQR: 936, 1620) was reported, with significant differences between men and women.

The main finding of this observation study in hip fracture patients was that, besides older age, even a moderate weight loss in hip fracture patients was associated with reduced mobility (NMS ≤ 5) at two months after the fracture. This association was independent of other factors including sex, energy or protein intake, and major comorbidities (cardiovascular disease, diabetes mellitus, hypertension, chronic obstructive pulmonary disease). However, observed weight change was less than 5% in most patients, and would therefore not be considered as a significant weight loss (which is usually set at 5%) (214).

6. Discussion

The effects of nutritional factors on age-related physiological changes including loss of muscle mass, strength, physical function, and bone mass, with the serious consequence of a hip fracture have been the overall theme of this thesis. In community-dwelling older persons, we found that vitamin D supplementation was not clinically relevant for muscle strength and mobility. Fish intake was positively associated with bone mineral density, but only in older women and not in the other groups. In older men, however, we observed an increased risk of hip fractures where the consumption of fish had been low. Thus, the association of fish intake with bone mineral density did not translate into a reduced risk of hip fractures in the group of older women. In the clinical study, it was observed that about ¾ of the hip fracture patients lost weight during follow-up, and 2 months after the fracture weight loss and age were negatively associated with mobility.

6.1 Methodological considerations

6.1.1 Study design and data analysis

In the hierarchy of research designs, meta-analyses of RCTs are regarded as the design providing the best evidence. However, there are many potential pitfalls associated with meta-analyses, ranging from completeness of the trawl through the literature to evaluation of individual studies and the statistical analysis. In the present case, we performed a systematic search of the literature in relevant medical databases, including the trial registry 'ClinicalTrials.gov', which allows identification of ongoing and therefore still unpublished trials. Relevant articles were also searched manually. Predefined inclusion and exclusion criteria and predefined search terms were used. We included only those RCTs in which vitamin D supplementation had been used. However, the vitamin D was provided in various forms, as vitamin D_2 and D_3 or as active metabolites such as $1,25\text{-}(OH)_2D$. In most studies there was also

concurrent supplementation of calcium in a variety of doses. In addition, the vitamin D supplementation was provided in daily, weekly, or monthly doses, and the route of administration was either oral or intramuscular. The duration of the studies also varied considerably. The outcomes of the included studies showed considerable differences, which made comparisons difficult and precluded a formal meta-analysis of outcomes other than TUG and HGS. The number of studies included in the meta-analysis was relatively small, and the heterogeneity of the studies was substantial.

The main strength of the meta-analysis design is the ability to pool small studies together. However, a meta-analysis based solely on small-scale studies has a lower capacity for critical bias evaluation, since the statistical tests require a large number of studies. Owing to the small number of studies included in the meta-analysis, it was difficult to perform a subgroup analysis that could explain the high degree of heterogeneity. The characteristics of baseline vitamin D status, oral administration of the supplement, the daily dose of vitamin D, the placebo group, supplementation with vitamin D_2 or D_3 or with active metabolites, and advice on calcium supplementation were all explored, but could not explain the heterogeneity. It would have been interesting to investigate the data using meta-regression, but this could not be done because of the small number of studies. Publication bias and heterogeneity could also have been explored using funnel plots (215), but again the lack of statistical power was an issue. This means that the results must be presented with circumspection (215).

In addition to the quantitative meta-analysis, we also conducted a narrative systematic review on the available evidence concerning the effects of vitamin D supplementation on muscle strength and mobility. After Paper I had been published, we identified two more studies that were relevant and that had been published recently (212, 213). These were high-quality studies of adequate scale, and they are described in the Results section. One of them (212) further confirmed the findings of the systematic review that vitamin D supplementation with or without calcium has little or no effect on muscle strength measurements. The other study (213) found a significant improvement in both muscle strength and mobility after supplementation

with the vitamin D metabolite α-calcidiol and with calcium. In comparison with the studies included in Paper I, these two studies differed in the vitamin D supplementation provided, in modes of administration (daily doses and bolus), in the outcome measures, and in duration. In one of them (212) over half of the participants were vitamin D- deficient, whereas in the other study (213) the number of vitamin D-deficient women was not stated.

In contrast to the positive findings of the new publication (213) in the majority of studies there was no evidence of any improvement in muscle strength or mobility after vitamin D supplementation. This could have been due to a number of reasons, for example because improvement of muscle function is more difficult at more advanced age and would require longer study periods. More promising effects has been observed in studies using interventions that combine nutritional measures and exercise (17). More standardized measurement of the outcome measures is highly recommended (216).

In Papers II and III we used data from the HUSK-study, in which the baseline examinations were performed in 1997-1999. Information on diet as the exposure variable was self-reported, with a validated FFQ (217, 218). Information on the outcome BMD was obtained by the DXA method, which is considered to be the gold standard (31). The outcome hip fracture was determined by an electronic search of patients' hospital records (196, 197, 219), ensuring high ascertainment of cases. Paper II analyzed cross-sectional data, while prospective data were used in Paper III. However, observational studies are not suitable for establishing causality.

In Papers II and III, the analyses were adjusted for a number of variables that were either measured or self-reported. Energy intake, alcohol consumption, and physical activity were all self-reported, and are thus subject to different biases. Recall of physical activity, diet, and alcohol consumption is difficult. BMI was calculated from measured height and weight, and is therefore a precise measure. Determination of plasma cotinine is more objective than self-reported smoking habits, as it measures

current tobacco use with good correlation, and even includes exposure to passive smoking (211).

In Paper IV a longitudinal design was applied. The hip fracture patients were followed up for approximately 2-3 months after the fracture. The primary endpoint was mobility at this time-point. The longitudinal design was suitable for tracking potential changes in energy intake and weight changes over time (220). In the immediate postoperative period the patients are in a catabolic state, and their basal energy needs are therefore increased (184), increasing the risk of a loss of weight.

All dietary interviews and anthropometric measurements were carried out directly, not using a proxy. Weight measurements were difficult to perform in the hospital shortly after surgery, as the patients were tired or in pain. No bed-scales or chair-scales were available, so that self-reported weights had to be used in about half of the cases. Mobility was assessed with the aid of the NMS, an easy and valid instrument (204). This method was chosen as it could be applied in the patients' homes and considered their physical limitations. In the logistic regression, age was used as the explaining variable in all models, and only one additional explaining variable was used concurrently because of the small number of patients.

6.1.2 Study population

In the present thesis the focus was solely on community-dwelling adults and older persons. By excluding nursing home residents it could be assumed that the comorbidity burden in the population would be reduced. However, even in community-dwelling older persons there is a high prevalence of comorbidity, which was also observed in the different populations investigated in the present thesis. This is evident by use of medications in the middle-aged HUSK participants (221), comorbidities in the older cohort (222), and the comorbidity burden in the clinical hip fracture study (data not shown). In addition, in many countries home care services allow older persons to live longer independently despite their diminished health

status. The reasons for focusing on this group of older persons were different in the different studies included in this thesis. In Paper I, the focus on community-dwelling older persons was in contrast to other studies that combined nursing home residents or hospitalized and community-dwelling older persons (140-145). Although this reduced heterogeneity among the participants, it did not reduce heterogeneity between the studies.

In HUSK, the participants were community-dwelling at baseline, but there was no information on whether they had moved into an institution after the baseline examination. In population-based epidemiologic studies the problem is that not all of those who are selected for inclusion are willing to take part. In a number of studies (223, 224), it was found that the socio-economic status and personal traits determine whether a person will be willing to participate. People with lower education and lower socio-economic status are as a rule less likely to participate, which impairs generalizability. Similar trends were observed in HUSK. Some information on the level of education and socio-economic status is available for the non-participants. The average income and the highest degree of education were higher among the participants than in the non- participants, and this applied to both men and women. In addition, significantly more of the non-participants were receiving social security benefits (194).

In HUSK, the participants were included as part of two birth cohorts with a small age range (40-43 and 70-74 years old), including both men and women. This is a difference from other studies on BMD and the risk of hip fracture, which were usually performed in older persons with a wider age range (age above 65 years) (166, 167) or in postmenopausal women (168, 170). Thus, age was not a factor to adjust for in the present analyses. BMD was stratified specifically for age and gender because of the loss of BMD over time. Though the study was cross-sectional, it was evident that BMD was much lower in the older cohorts than in the middle-aged, and this was particularly evident in women.

The hip fracture study also included community-dwelling older persons, but (as in the other studies) it excluded patients with dementia or cognitive decline. More specifically, all patients unable to give their informed consent were excluded as a proxy for dementia. By choosing to include only community-dwelling persons without dementia, the "healthiest" of the hip fracture group were the target and those with a high comorbidity burden were excluded (225). Still, both the recruitment and the rate of dropouts from follow-up presented great difficulties. This is similar to the situation in other studies on older persons and patients (66, 131, 185, 226), and it may have compromised both external and internal validity.

6.1.3 Methods of dietary intake assessment

As stated by the late Professor George Beaton (227): "There will always be errors in dietary assessments. The problem is to understand, estimate, and make use of the error structure during analysis". This is the very essence of dealing with dietary intake data. Both the FFQ and the 24HR were used in the present thesis. These methods are not directly comparable and they lead to different results.

Food frequency questionnaire (FFQ)

The FFQ used in HUSK was a 169-item semi-quantitative questionnaire. The strengths of the FFQ are that it is easy to fill out for the participants, it is an easy and cost-efficient way of assessing dietary habits in large cohorts, and it reflects habitual diets. Its limitations include estimation of the portion sizes and the non-reporting of food items that are not listed. Both of these limitations contribute to underreporting (228). However, it has been shown that the energy intake increases with increasing number of items to choose from, and that it would be difficult to obtain correct estimates from foods consumed only in specific seasons. Nevertheless, HUSK used a 169-item FFQ, which provided many food items to choose from, was conducted all year round, and the large number of participants may have offset this problem.

Intake of fish was the exposure variable explored in both Papers II and III. Knowledge of the fish intake in the general Norwegian population in 1997-1999 (when HUSK was carried out) can be compared with the national dietary survey NORKOST2 (117), which also used an FFQ, although a different. The fish intake in HUSK is actually overall comparable with the fish intake in NORKOST2, which may support the external validity of the HUSK data. The FFQ listed food items in a way that was adapted to Norwegian consumption habits. This is illustrated by the Norwegian habit of consuming fish as spread and the use of several fish products eaten for dinner, which is very specific for the Norwegian diet (229).

A small, but significant positive effect of fish consumption on BMD was observed in older women in Paper II. In Paper III a low fish intake was associated with an increased risk of hip fracture, especially in older men. The measurement errors, which often occur in FFQ data, may cause associations to be under- or overestimated. This is in addition to occurrence of other residual confounding that is often found e.g. in a regression model (228). In the present dietary data, several measures were taken to reduce the errors of the dietary intake determinations. In the first place, the questionnaire was compared against another method of dietary intake assessment (weighted dietary records) (217) and against a biomarker (fatty acid composition in serum phospholipids and in adipose tissue) (217, 218, 230). The FFQ has been validated specifically for fish and n3 PUFAs intake (217, 218). The overall correlation coefficients between docosahexaenoic acid (DHA) intake according to the FFQ and the DHA content of adipose tissue and serum phospholipids were both 0.49, which is in line with the results of several similar investigations (231-233). Secondly, the analysis in Papers II and III was energy-adjusted (234), which may improve the risk estimate of low BMD and the risk of hip fractures (228). Despite these measures, misclassification cannot be ruled out. The energy intakes in persons after hip fracture observed during follow-up and in persons without fracture were similar, despite different mean BMI (data not shown). This may indicate underreporting, especially in subjects with higher BMIs; this is a common observation in dietary studies (235).

24-hour recall (24HR)

In Paper IV, the dietary intake was assessed by the 24HR method. The strengths of this method are that it is easy to perform both for the respondent and for a trained interviewer; it is quick, it gives more detailed information on the foods consumed than an FFQ, it is culturally neutral, and it tends to be less prone to biases compared with the FFQ (236). The primary limitations of the 24HR are problems with remembering the food intake over last 24 hours and quantification of the food eaten (228). This makes the 24HR prone to underreporting that also may have been relevant in the present study. Underreporting by the 24HR can be reduced by using standardized methods, e.g. the multiple pass method that has also been used in the present study (198, 237). Severalfold repetition of the 24HR has been shown to give a better estimate of both energy intake and nutrients (238). However, a Swedish study that investigated predictors of underreporting using the multiple pass method and repetitions of the 24HR still reported that BMI, old age and smoking were the main predictors of underreporting (239). We have reason to believe that underreporting occurred also in the present study. At visit 3, the median ratio between calculated energy intake and resting metabolic rate was 1.3, which is rather low. Furthermore, it can be speculated that if the patients included had consumed only the reported amounts of energy, the weight loss must have been even more pronounced than the observed one. However, the hip fracture patients included in this study would not have been able to complete an FFQ or dietary records, at least not during hospitalization or rehabilitation, and therefor the 24HR was chosen for dietary assessment.

6.2 Discussion of general aspects of the thesis

6.2.1 Food and supplements

The present thesis investigated both the effects of dietary supplements and fish as food group on various health outcomes. In intervention studies, for both scientific and practical reasons it is much easier to conduct a study with dietary supplements than with actual food. Issues may include blinding of the investigators and the study participants, standardization, bioavailability, taste preferences, daily life suitability, and energy compensation. This may be why there are many more intervention studies with supplements than with specific food items. This is illustrated by the low number of RCTs using fish as interventions with intermediate outcomes (101, 218). However, even in supplementation trials there are problems, concerning the duration of supplementation in comparison with lifelong consumption of a food item, the doses, and in the case of vitamin D the metabolites and the route of administration. These challenges were evident in our meta-analysis of vitamin D supplementation and muscle strength and mobility (Paper I) as was indicated by the high degree of heterogeneity between studies. Changes in health outcomes such as muscle strength, mobility and BMD usually do not occur quickly, but develop slowly over time. This means that the habitual diet and lifestyle have a greater effect on these outcomes than supplementation administered for a short period of time (240). However, supplementation use should not be ruled out, but may have a place in addition to the habitual diet. However, supplementation should be evaluated according to the participants' deficiency/sufficiency status, the age, the total energy intake and the dietary quality. As an illustration, vitamin D and calcium supplementation is recommended to prevent low BMD to those with low vitamin D and calcium intake (241). Another issue for comparison of food and supplementation is the case of fish and n3 PUFAs. Opposed to the many intervention studies on health effects of n3 PUFAs, there are only limited number of intervention studies on fish with clinical outcomes. As an illustration, to the best of our knowledge, we did not find an intervention with fish for the prevention of sarcopenia. However, RCTs using n3 PUFAs have been conducted in primary prevention of sarcopenia and have

reported positive results (242-244). Even further, n3 PUFA supplementation also had positive effects on muscle mass in sarcopenic older persons (245). Thus, it has to be shown whether an intervention with fish would have similar results.

6.2.2 Predictors for preserving muscle and bone mass with aging

Both loss of muscle mass (sarcopenia) and loss of bone mass (osteoporosis) are conditions that are part of the aging process. Although the reasons for the reduction of muscle and bone mass are multifactorial, they have several factors in common, including reduced physical activity, reduction of sex hormones, and catabolic stimuli due to chronic inflammation (246). The loss of muscle mass has implications for muscle strength and mobility, often indicated by an increased risk of falls (247, 248). The loss of bone mass leads to an elevated risk of low-energy fractures. Older persons suffering from both sarcopenia and osteoporosis are at an increased risk of a fall and fracture (249), and the term osteosarcopenia recently has been introduced (250) for these particularly frail individuals. Not surprisingly, the prevalence of sarcopenia is high in hip fracture patients, ranging from 17- 58% using the EWGSOP definition on sarcopenia (251-253).

Among the many risk factors of osteoporosis and sarcopenia, some are unmodifiable. Low BMD is associated with female gender (30), ethnicity (254, 255), familial history of osteoporosis (256, 257) and genetic factors, which account for about 70 % of BMD variability (33, 258). In muscle mass, the genetic implications and sex differences are also evident, and, similarly as in BMD, a high proportion of the variability in lean mass is thought to be explained by genetic factors (33). In both low BMD and muscle mass, the modifiable risk factors, accounting for only some 20-40% of the variability. These include smoking (27, 259), little physical activity (26, 260), and the diet (33, 49). Thus, diet plays a limited, but important, role in the prevention of low BMD and muscle mass. Since a low BMD is one of the strongest risk factors for hip fractures (48), the above-mentioned factors are relevant for hip fracture prevention as well. In both preserving muscle and bone mass, a combination

of nutritional factors and physical activity seems to be the key modifiable factors (33, 261). Physical activity is an important measure owing to its stimulation of protein synthesis in muscles (24) and mechanical loading that promotes bone strength (260, 262). Physical activity also increases the energy requirement and appetite (263), and is therefore associated with higher dietary intake, which is positive in this regard.

Bearing in mind that adequate energy intake and quality of the diet make an important contribution to healthy aging, it is astonishing that dietary recommendations so far have not been focused particularly on very old persons (older than 80 years). Owing to the age-dependent muscle loss and the reduced physical activity, the energy requirements in healthy older persons are decreased compared to younger adults. Owing to anabolic resistance, the utilization of dietary protein in protein synthesis and the counteractive effect on muscle degradation is reduced (24), which indicates higher protein requirements (264). The European Society for Clinical Nutrition and Metabolism (120) has already suggested higher protein recommendations for older persons but this has not been considered in national recommendations. In the NNR5 (91), the lower limit of protein recommendation is higher for older than for younger persons, but at the same time, it is expressed that there is huge uncertainty on the protein requirements in older persons.

In contrast to energy requirements, those for vitamins and minerals, however, remain the same or increase. This is due to a number of physiological changes, such as the reduced absorption in the gastrointestinal tract, reduced number of parietal cells in the stomach, and less effective skin synthesis of vitamin D (75). In the case of vitamin D, there are already recommendations for older persons (**Table 4**), which refer to both changed physiology and increased risk of fractures.

At present, there is huge uncertainty about the nutritional needs for very old persons. Their requirements are further affected by the high burden of chronic diseases and medication use. This is a field of research with extensive knowledge-gap. There is a need for more research in the health effects of diet in older persons.

7. Conclusion

Community-dwelling older persons did not benefit from vitamin D supplementation in terms of muscle strength and mobility. In older women, high fish intake seems to have beneficial effects on BMD that, however did not translate into reduced risk of hip fractures. One possible explanation could be that other factors are more important than fish intake in this cohort of well-nourished, community-dwelling old women. Although we observed a small risk increase of hip fractures in old men who were in the lowest quartile of fish consumption, these results should be interpreted with caution, as we did not see any effect on BMD in men and no association of fish intake with hip fracture risk in women. Thus, a chance finding cannot be excluded.

The most important conclusions from the clinical study are first, that older persons admitted to the hospital are difficult to motivate to participate in a clinical study. This is shown by the relative low number of patients and the high attrition rate. Secondly, hip fracture patients are a high-risk group of developing undernutrition. About ¾ of the included patients lost weight during the first three months post fracture, which was negatively associated with mobility at this time point.

Overall, there is a need for more high quality studies of the association of health outcomes and diet in community-dwelling older persons. This requires joint efforts from health professionals including clinical dietitians, physiotherapists, physicians and geriatrists.

8. Further perspective

Loss of muscle mass is one of the characteristics of aging. Low physical activity, low dietary quality, smoking and polypharmacy are among the factors that accelerate this process and thereby the risk of developing sarcopenia. Acute and chronic diseases that are common in an aging population also contribute to this development. Thus, future studies should include patients with chronic diseases such as cardiovascular disease, cancer, chronic obstructive pulmonary disease, and osteoporosis and investigate the prevalence of sarcopenia. Furthermore, determinants of loss of muscle mass and treatment options to maintain muscle mass in older persons should be investigated. Promising prevention and treatment interventions involve dietary (adequate energy and protein intake), lifestyle (physical activity and smoking cessation) and pharmaceutical (prevention of polypharmacy) measures.

As argued in the present thesis, there is uncertainty concerning the dietary recommendations for older persons. There is an extensive knowledge-gap for the dietary needs of this population group. To fill this gap, it requires joint efforts form the scientific community in collaboration with those working in primary and secondary health care.

9. Referances

1. Narici MV, Maffulli N. Sarcopenia: characteristics, mechanisms and functional significance. British medical bulletin. 2010;95:139-59.

2. WHO. World Report on Ageing and Health. Geneve, Switzerland: The World Health Organization; 2015.

3. Kinsella K, Wan H. International Population Reports, P95/09-1, An Aging World: 2008. Washington, DC: US Census Bureau; 2009.

4. Statistics Norway. Delen av befolkninga som er 65 år eller eldre. Utvalde land 2017 [Available from: http://www.ssb.no/318600/delen-av-befolkninga-som-er-65-ar-eller-eldre.utvalde-land-sa-107.

5. WHO. Fact sheet N°404: Ageing and Health: World Health Organization; 2015 [updated September 2015. Available from: http://www.who.int/mediacentre/factsheets/fs404/en/.

6. Lopez-Otin C, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. Cell. 2013;153(6):1194-217.

7. Langley-Evans S. Nutrition, ageing and the elderly. In: 2, editor. Nutrition, Health and Disease: A Lifespan Approach: John Wiley & Sons, Ltd.; 2015. p. 268-99.

8. Epidemiologic and methodologic problems in determining nutritional status of older persons. Proceedings of a conference. Albuquerque, New Mexico, October 19-21, 1988. The American journal of clinical nutrition. 1989;50(5 Suppl):1121-235.

9. Janssen I, Heymsfield SB, Ross R. Low relative skeletal muscle mass (sarcopenia) in older persons is associated with functional impairment and physical disability. Journal of the American Geriatrics Society. 2002;50(5):889-96.

10. Cruz-Jentoft AJ, Baeyens JP, Bauer JM, Boirie Y, Cederholm T, Landi F, et al. Sarcopenia: European consensus on definition and diagnosis: Report of the European Working Group on Sarcopenia in Older People. Age and ageing. 2010;39(4):412-23.

11. Baumgartner RN, Koehler KM, Gallagher D, Romero L, Heymsfield SB, Ross RR, et al. Epidemiology of sarcopenia among the elderly in New Mexico. Am J Epidemiol. 1998;147(8):755-63.

12.   Pichard C, Kyle UG, Bracco D, Slosman DO, Morabia A, Schutz Y. Reference values of fat-free and fat masses by bioelectrical impedance analysis in 3393 healthy subjects. Nutrition (Burbank, Los Angeles County, Calif). 2000;16(4):245-54.

13.   Fielding RA, Vellas B, Evans WJ, Bhasin S, Morley JE, Newman AB, et al. Sarcopenia: an undiagnosed condition in older adults. Current consensus definition: prevalence, etiology, and consequences. International working group on sarcopenia. Journal of the American Medical Directors Association. 2011;12(4):249-56.

14.   Cao L, Morley JE. Sarcopenia Is Recognized as an Independent Condition by an International Classification of Disease, Tenth Revision, Clinical Modification (ICD-10-CM) Code. Journal of the American Medical Directors Association. 2016;17(8):675-7.

15.   Woo J, Leung J, Morley JE. Validating the SARC-F: A Suitable Community Screening Tool for Sarcopenia? Journal of the American Medical Directors Association.15(9):630-4.

16.   Morley JE. Sarcopenia in the elderly. Family practice. 2012;29 Suppl 1:i44-i8.

17.   Cruz-Jentoft AJ, Landi F, Schneider SM, Zuniga C, Arai H, Boirie Y, et al. Prevalence of and interventions for sarcopenia in ageing adults: a systematic review. Report of the International Sarcopenia Initiative (EWGSOP and IWGS). Age and ageing. 2014;43(6):748-59.

18.   Hall JE, Guyton AC. Textbook of Medical Physiology. 12 ed. U.S.: Saunders Elsevier; 2011. 1091 p.

19.   Lexell J, Henriksson-Larsen K, Winblad B, Sjostrom M. Distribution of different fiber types in human skeletal muscles: effects of aging studied in whole muscle cross sections. Muscle Nerve. 1983;6(8):588-95.

20.   Dawson-Hughes B. Vitamin D and muscle function. The Journal of steroid biochemistry and molecular biology. 2017;173:313-6.

21.   Verdijk LB, Koopman R, Schaart G, Meijer K, Savelberg HH, van Loon LJ. Satellite cell content is specifically reduced in type II skeletal muscle fibers in the elderly. American journal of physiology Endocrinology and metabolism. 2007;292(1):E151-7.

22.   Brown WF. A method for estimating the number of motor units in thenar muscles and the changes in motor unit count with ageing. Journal of neurology, neurosurgery, and psychiatry. 1972;35(6):845-52.

23.   Rooyackers OE, Adey DB, Ades PA, Nair KS. Effect of age on in vivo rates of mitochondrial protein synthesis in human skeletal muscle. Proceedings of the National Academy of Sciences of the United States of America. 1996;93(26):15364-9.

24.   Boirie Y. Physiopathological mechanism of sarcopenia. The journal of nutrition, health & aging. 2009;13(8):717-23.

25.   Cuthbertson D, Smith K, Babraj J, Leese G, Waddell T, Atherton P, et al. Anabolic signaling deficits underlie amino acid resistance of wasting, aging muscle. FASEB journal : official publication of the Federation of American Societies for Experimental Biology. 2005;19(3):422-4.

26.   Sayer AA, Robinson SM, Patel HP, Shavlakadze T, Cooper C, Grounds MD. New horizons in the pathogenesis, diagnosis and management of sarcopenia. Age and ageing. 2013;42(2):145-50.

27.   Castillo EM, Goodman-Gruen D, Kritz-Silverstein D, Morton DJ, Wingard DL, Barrett-Connor E. Sarcopenia in elderly men and women: the Rancho Bernardo study. American journal of preventive medicine. 2003;25(3):226-31.

28.   Cosqueric G, Sebag A, Ducolombier C, Thomas C, Piette F, Weill-Engerer S. Sarcopenia is predictive of nosocomial infection in care of the elderly. The British journal of nutrition. 2006;96(5):895-901.

29.   WHO Study Group. Assessment of fracture risk and its application to screening for postmenopausal osteoporosis. . 1994.

30.   Kanis JA, Melton LJ, 3rd, Christiansen C, Johnston CC, Khaltaev N. The diagnosis of osteoporosis. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research. 1994;9(8):1137-41.

31.   Hoiberg MP, Rubin KH, Hermann AP, Brixen K, Abrahamsen B. Diagnostic devices for osteoporosis in the general population: A systematic review. Bone. 2016;92:58-69.

32.   Kanis JA, McCloskey EV, Johansson H, Oden A, Melton LJ, 3rd, Khaltaev N. A reference standard for the description of osteoporosis. Bone. 2008;42(3):467-75.

33.   Weaver CM, Gordon CM, Janz KF, Kalkwarf HJ, Lappe JM, Lewis R, et al. The National Osteoporosis Foundation's position statement on peak bone mass development and lifestyle factors: a systematic review and implementation recommendations. Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA. 2016.

34.   Cooper C, Campion G, Melton LJ, 3rd. Hip fractures in the elderly: a world-wide projection. Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA. 1992;2(6):285-9.

35.   Lunt M, Felsenberg D, Adams J, Benevolenskaya L, Cannata J, Dequeker J, et al. Population-based geographic variations in DXA bone density in Europe: the EVOS Study. European Vertebral Osteoporosis. Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA. 1997;7(3):175-89.

36.   Sambrook P, Cooper C. Osteoporosis. Lancet. 2006;367(9527):2010-8.

37.   Seeley R, VanPutte C, Regan J, Russo A. Seeley's Anatomy and Physiology 9ed. New York, US: McGraw-Hill; 2011.

38.   Lieben L, Carmeliet G. Vitamin D signaling in osteocytes: effects on bone and mineral homeostasis. Bone. 2013;54(2):237-43.

39.   Koh JM, Khang YH, Jung CH, Bae S, Kim DJ, Chung YE, et al. Higher circulating hsCRP levels are associated with lower bone mineral density in healthy pre- and postmenopausal women: evidence for a link between systemic inflammation and osteoporosis. Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA. 2005;16(10):1263-71.

40.   Khosla S, Melton LJ, Riggs BL. The unitary model for estrogen deficiency and the pathogenesis of osteoporosis: is a revision needed? Journal of bone and mineral

research : the official journal of the American Society for Bone and Mineral Research. 2011;26(3):441-51.

41. Mundy GR. Osteoporosis and inflammation. Nutrition reviews. 2007;65(12 Pt 2):S147-51.

42. Albright F, Smith PH, Richardson AM. Postmenopausal osteoporosis: Its clinical features. Journal of the American Medical Association. 1941;116(22):2465-74.

43. Riggs BL, Melton LJ, 3rd. Evidence for two distinct syndromes of involutional osteoporosis. The American journal of medicine. 1983;75(6):899-901.

44. Manson JE, Chlebowski RT, Stefanick ML, Aragaki AK, Rossouw JE, Prentice RL, et al. Menopausal hormone therapy and health outcomes during the intervention and extended poststopping phases of the Women's Health Initiative randomized trials. JAMA : the journal of the American Medical Association. 2013;310(13):1353-68.

45. Fink HA, Ewing SK, Ensrud KE, Barrett-Connor E, Taylor BC, Cauley JA, et al. Association of testosterone and estradiol deficiency with osteoporosis and rapid bone loss in older men. The Journal of clinical endocrinology and metabolism. 2006;91(10):3908-15.

46. Kanis JA, Johnell O, De Laet C, Johansson H, Oden A, Delmas P, et al. A meta-analysis of previous fracture and subsequent fracture risk. Bone. 2004;35(2):375-82.

47. Omsland TK, Emaus N, Tell GS, Ahmed LA, Center JR, Nguyen ND, et al. Ten-year risk of second hip fracture. A NOREPOS study. Bone. 2013;52(1):493-7.

48. Cummings SR, Melton LJ. Epidemiology and outcomes of osteoporotic fractures. Lancet. 2002;359(9319):1761-7.

49. Cauley JA. Osteoporosis: fracture epidemiology update 2016. Current opinion in rheumatology. 2017;29(2):150-6.

50. Diaz de Bustamante M, Alarcon T, Menendez-Colino R, Ramirez-Martin R, Otero A, Gonzalez-Montalvo JI. Prevalence of malnutrition in a cohort of 509 patients with acute hip fracture: the importance of a comprehensive assessment. European journal of clinical nutrition. 2017.

51. Meyer HE, Tverdal A, Falch JA. Risk factors for hip fracture in middle-aged Norwegian women and men. Am J Epidemiol. 1993;137(11):1203-11.

52. Meyer HE, Tverdal A, Falch JA. Body height, body mass index, and fatal hip fractures: 16 years' follow-up of 674,000 Norwegian women and men. Epidemiology (Cambridge, Mass). 1995;6(3):299-305.

53. Norwegian Hip Fracture Register. Annual report 2016: Norwegian National Advisory Unit on Arthroplasty and Hip Fractures. 2016.

54. Omsland TK, Gjesdal CG, Emaus N, Tell GS, Meyer HE. Regional differences in hip bone mineral density levels in Norway: the NOREPOS study. Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA. 2009;20(4):631-8.

55. Omsland TK, Holvik K, Meyer HE, Center JR, Emaus N, Tell GS, et al. Hip fractures in Norway 1999-2008: time trends in total incidence and second hip fracture rates: a NOREPOS study. Eur J Epidemiol. 2012;27(10):807-14.

56. Gronskag AB, Romundstad P, Forsmo S, Langhammer A, Schei B. Excess mortality after hip fracture among elderly women in Norway. The HUNT study. Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA. 2012;23(6):1807-11.

57. Vochteloo AJ, Moerman S, Tuinebreijer WE, Maier AB, de Vries MR, Bloem RM, et al. More than half of hip fracture patients do not regain mobility in the first postoperative year. Geriatr Gerontol Int. 2013;13(2):334-41.

58. Folbert EC, Hegeman JH, Gierveld R, van Netten JJ, Velde DV, Ten Duis HJ, et al. Complications during hospitalization and risk factors in elderly patients with hip fracture following integrated orthogeriatric treatment. Archives of orthopaedic and trauma surgery. 2017;137(4):507-15.

59. Meyer HE, Tverdal A, Falch JA, Pedersen JI. Factors associated with mortality after hip fracture. Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA. 2000;11(3):228-32.

60.   Lee HB, Oldham MA, Sieber FE, Oh ES. Impact of Delirium After Hip Fracture Surgery on One-Year Mortality in Patients With or Without Dementia: A Case of Effect Modification. The American journal of geriatric psychiatry : official journal of the American Association for Geriatric Psychiatry. 2017;25(3):308-15.

61.   Holvik K, Ranhoff AH, Martinsen MI, Solheim LF. Predictors of mortality in older hip fracture inpatients admitted to an orthogeriatric unit in oslo, norway. Journal of aging and health. 2010;22(8):1114-31.

62.   Cauley JA, Cawthon PM, Peters KE, Cummings SR, Ensrud KE, Bauer DC, et al. Risk Factors for Hip Fracture in Older Men: The Osteoporotic Fractures in Men Study (MrOS). Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research. 2016;31(10):1810-9.

63.   Heikkinen T, Jalovaara P. Four or twelve months' follow-up in the evaluation of functional outcome after hip fracture surgery? Scandinavian journal of surgery : SJS : official organ for the Finnish Surgical Society and the Scandinavian Surgical Society. 2005;94(1):59-66.

64.   Magaziner J, Simonsick EM, Kashner TM, Hebel JR, Kenzora JE. Predictors of functional recovery one year following hospital discharge for hip fracture: a prospective study. Journal of gerontology. 1990;45(3):M101-7.

65.   Miller CW. Survival and ambulation following hip fracture. J Bone Joint Surg Am. 1978;60(7):930-4.

66.   Steihaug OM, Gjesdal CG, Bogen B, Kristoffersen MH, Lien G, Hufthammer KO, et al. Does sarcopenia predict change in mobility after hip fracture? a multicenter observational study with one-year follow-up. BMC geriatrics. 2018;18(1):65.

67.   Ross AC, Taylor CL, Yaktine AL, Institute of Medicine (US). Committee to Review Dietary Reference Intakes for Vitamin D and Calcium. Dietary Reference Intakes for Calcium and Vitamin D. Washington (DC): National Academies Press (US); 2011.

68.   Simon RR, Phillips KM, Horst RL, Munro IC. Vitamin D mushrooms: comparison of the composition of button mushrooms (Agaricus bisporus) treated postharvest with UVB light or sunlight. Journal of agricultural and food chemistry. 2011;59(16):8724-32.

69.   Lamberg-Allardt C. Vitamin D in foods and as supplements. Progress in biophysics and molecular biology. 2006;92(1):33-8.

70.   Totland TH, Melnæs BK, Lundberg-Hallén N, Helland-Kigen KM, Lund-Blix NA, Myhre JB, et al. Norkost 3 - A National Dietary Survey in among adults aged 18-70 years in Norway, 2010-11. 2012.

71.   Zgaga L, Theodoratou E, Farrington SM, Agakov F, Tenesa A, Walker M, et al. Diet, environmental factors, and lifestyle underlie the high prevalence of vitamin D deficiency in healthy adults in Scotland, and supplementation reduces the proportion that are severely deficient. The Journal of nutrition. 2011;141(8):1535-42.

72.   Holick MF. Vitamin D: a D-Lightful health perspective. Nutrition reviews. 2008;66(10 Suppl 2):S182-94.

73.   Engelsen O. The relationship between ultraviolet radiation exposure and vitamin D status. Nutrients. 2010;2(5):482-95.

74.   Wacker M, Holick MF. Sunlight and Vitamin D: A global perspective for health. Dermato-endocrinology. 2013;5(1):51-108.

75.   Holick MF, Matsuoka LY, Wortsman J. Age, vitamin D, and solar ultraviolet. Lancet. 1989;2(8671):1104-5.

76.   Yamamoto M, Kawanobe Y, Takahashi H, Shimazawa E, Kimura S, Ogata E. Vitamin D Deficiency and Renal Calcium Transport in the Rat. The Journal of clinical investigation. 1984;74(August):507-13.

77.   Holick MF. Vitamin D deficiency. The New England journal of medicine. 2007;357(3):266-81.

78.   Lips P. Vitamin D physiology. Progress in biophysics and molecular biology. 2006;92(1):4-8.

79.   Haussler MR, Whitfield GK, Kaneko I, Haussler CA, Hsieh D, Hsieh JC, et al. Molecular mechanisms of vitamin D action. Calcified tissue international. 2013;92(2):77-98.

80.   Erben RG, Andrukhova O. FGF23-Klotho signaling axis in the kidney. Bone. 2017;100:62-8.

81.   Boland R. Role of vitamin D in skeletal muscle function. Endocrine reviews. 1986;7(4):434-48.

82.   Bischoff-Ferrari HA, Giovannucci E, Willett WC, Dietrich T, Dawson-Hughes B. Estimation of optimal serum concentrations of 25-hydroxyvitamin D for multiple health outcomes. The American journal of clinical nutrition. 2006;84(1):18-28.

83.   Bischoff HA, Stahelin HB, Urscheler N, Ehrsam R, Vonthein R, Perrig-Chiello P, et al. Muscle strength in the elderly: its relation to vitamin D metabolites. Archives of physical medicine and rehabilitation. 1999;80(1):54-8.

84.   Mowe M, Haug E, Bohmer T. Low serum calcidiol concentration in older adults with reduced muscular function. Journal of the American Geriatrics Society. 1999;47(2):220-6.

85.   Schott GD, Wills MR. Muscle weakness in osteomalacia. Lancet. 1976;1(7960):626-9.

86.   De Boland AR, Boland RL. Non-genomic signal transduction pathway of vitamin D in muscle. Cellular signalling. 1994;6(7):717-24.

87.   Girgis CM, Clifton-Bligh RJ, Hamrick MW, Holick MF, Gunton JE. The roles of vitamin D in skeletal muscle: form, function, and metabolism. Endocrine reviews. 2013;34(1):33-83.

88.   Simpson RU, Thomas GA, Arnold AJ. Identification of 1,25-dihydroxyvitamin D3 receptors and activities in muscle. The Journal of biological chemistry. 1985;260(15):8882-91.

89.   Windelinckx A, De Mars G, Beunen G, Aerssens J, Delecluse C, Lefevre J, et al. Polymorphisms in the vitamin D receptor gene are associated with muscle strength in men and women. Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA. 2007;18(9):1235-42.

90.   Bischoff-Ferrari HA, Borchers M, Gudat F, Durmuller U, Stahelin HB, Dick W. Vitamin D receptor expression in human muscle tissue decreases with age. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research. 2004;19(2):265-9.

91.   Nordic Council of Ministers. Nordic Nutritional Recommendations 2012 - Integrating nutrition and physical activity. 2012.

92.   Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. The Journal of clinical endocrinology and metabolism. 2011;96(7):1911-30.

93.   Hypponen E, Power C. Hypovitaminosis D in British adults at age 45 y: nationwide cohort study of dietary and lifestyle predictors. The American journal of clinical nutrition. 2007;85(3):860-8.

94.   Cashman KD, Dowling KG, Skrabakova Z, Gonzalez-Gross M, Valtuena J, De Henauw S, et al. Vitamin D deficiency in Europe: pandemic? The American journal of clinical nutrition. 2016;103(4):1033-44.

95.   Cashman KD, Ritz C, Kiely M, Odin C. Improved Dietary Guidelines for Vitamin D: Application of Individual Participant Data (IPD)-Level Meta-Regression Analyses. Nutrients. 2017;9(5).

96.   Cashman KD, Kiely M. Recommended dietary intakes for vitamin D: Where do they come from, what do they achieve and how can we meet them? J Hum Nutr Diet. 2014;27(5):434-42.

97.   Efsa Panel on Dietetic Products, Nutrition and Allergies. Scientific Opinion on the Tolerable Upper Intake Level of vitamin D. EFSA Journal. 2012;10(7):2813-n/a.

98.   NIH. Vitamin D - Fact sheet for health professionals: National Institutes of Health; 2016 [updated 11.02.2016. Available from: https://ods.od.nih.gov/factsheets/VitaminD-HealthProfessional/.

99.   EFSA. Scientific Opinion on Dietary Reference Values for vitamin D. Parma, Italy: European Food Safety Authority; 2016.

100.   New reference values for vitamin D. Ann Nutr Metab. 2012;60(4):241-6.

101.   Lehmann U, Gjessing HR, Hirche F, Mueller-Belecke A, Gudbrandsen OA, Ueland PM, et al. Efficacy of fish intake on vitamin D status: a meta-analysis of randomized controlled trials. The American journal of clinical nutrition. 2015;102(4):837-47.

102.   VKM. A comprehensive assessment of fish and other seafood in the Norwegian diet. The Norwegian Scientific Committee for Food Safety; 2006.

103.   Welch AA, Lund E, Amiano P, Dorronsoro M, Brustad M, Kumle M, et al. Variability of fish consumption within the 10 European countries participating in the European Investigation into Cancer and Nutrition (EPIC) study. Public Health Nutr. 2002;5(6b):1273-85.

104.   Varela-Moreiras G, Avila JM, Cuadrado C, del Pozo S, Ruiz E, Moreiras O. Evaluation of food consumption and dietary patterns in Spain by the Food Consumption Survey: updated information. European journal of clinical nutrition. 2010;64 Suppl 3:S37-43.

105.   National Institute for Public Health and the Environment. Dutch National Food Consumption Survey 2007– 2010. Bilthoven: RIVM; 2016.

106.   Heuer T, Krems C, Moon K, Brombach C, Hoffmann I. Food consumption of adults in Germany: results of the German National Nutrition Survey II based on diet history interviews. The British journal of nutrition. 2015;113(10):1603-14.

107.   DOH. Anbefalinger om kosthold, ernæring og fysisk aktivitet. Oslo, Norway: The Norwegian Directorat of Health; 2014.

108.   Kromhout D, Spaaij CJ, de Goede J, Weggemans RM. The 2015 Dutch food-based dietary guidelines. European journal of clinical nutrition. 2016;70(8):869-78.

109.   Zheng J, Huang T, Yu Y, Hu X, Yang B, Li D. Fish consumption and CHD mortality: an updated meta-analysis of seventeen cohort studies. Public Health Nutr. 2012;15(4):725-37.

110.   Xun P, Qin B, Song Y, Nakamura Y, Kurth T, Yaemsiri S, et al. Fish consumption and risk of stroke and its subtypes: accumulative evidence from a meta-analysis of prospective cohort studies. European journal of clinical nutrition. 2012;66(11):1199-207.

111.   de Roos B, Sneddon AA, Sprague M, Horgan GW, Brouwer IA. The potential impact of compositional changes in farmed fish on its health-giving properties: is it time to reconsider current dietary recommendations? Public Health Nutr. 2017:1-8.

112.   Scientific Advisory Committee on Nutrition. Advise on fish consumption: benefits and risks. London; 2004.

113.   Sirot V, Leblanc JC, Margaritis I. A risk-benefit analysis approach to seafood intake to determine optimal consumption. The British journal of nutrition. 2012;107(12):1812-22.

114.   VKM. Benefit-risk assessment of fish and fish products in the Norwegian diet-an update. Opinion of the Scientific Steering Committee of the Norwegian Scientific Committee for Food Safety; 2014.

115.   Lund EK. Health benefits of seafood; is it just the fatty acids? Food chemistry. 2013;140(3):413-20.

116.   Zhang YF, Gao HF, Hou AJ, Zhou YH. Effect of omega-3 fatty acid supplementation on cancer incidence, non-vascular death, and total mortality: a meta-analysis of randomized controlled trials. BMC Public Health. 2014;14:204.

117.   Johansson L, Solvoll K. Norkost 1997 - Landsomfattende kostholdsundersøkelse blant menn og kvinner i alderen 16-79 år.: Statens råd for ernæring og fysisk aktivitet.; 1999.

118.   Rosendahl-Riise H, Karlsson T, Drevon CA, Apalset EM, Nygard OK, Tell GS, et al. Total and lean fish intake is positively associated with bone mineral density in older women in the community-based Hordaland Health Study. European journal of nutrition. 2018.

119.   Mithal A, Bonjour JP, Boonen S, Burckhardt P, Degens H, El Hajj Fuleihan G, et al. Impact of nutrition on muscle mass, strength, and performance in older adults. Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA. 2013;24(5):1555-66.

120.   Deutz NE, Bauer JM, Barazzoni R, Biolo G, Boirie Y, Bosy-Westphal A, et al. Protein intake and exercise for optimal muscle function with aging: recommendations from the ESPEN Expert Group. Clinical nutrition (Edinburgh, Scotland). 2014;33(6):929-36.

121.   McLean RR, Mangano KM, Hannan MT, Kiel DP, Sahni S. Dietary Protein Intake Is Protective Against Loss of Grip Strength Among Older Adults in the Framingham Offspring Cohort. The journals of gerontology Series A, Biological sciences and medical sciences. 2016;71(3):356-61.

122.   Beasley JM, Wertheim BC, LaCroix AZ, Prentice RL, Neuhouser ML, Tinker LF, et al. Biomarker-calibrated protein intake and physical function in the Women's Health Initiative. Journal of the American Geriatrics Society. 2013;61(11):1863-71.

123.   Chan R, Leung J, Woo J, Kwok T. Associations of dietary protein intake on subsequent decline in muscle mass and physical functions over four years in ambulant older Chinese people. The journal of nutrition, health & aging. 2014;18(2):171-7.

124.   Gregorio L, Brindisi J, Kleppinger A, Sullivan R, Mangano KM, Bihuniak JD, et al. Adequate dietary protein is associated with better physical performance among post-menopausal women 60-90 years. The journal of nutrition, health & aging. 2014;18(2):155-60.

125.   Isanejad M, Mursu J, Sirola J, Kroger H, Rikkonen T, Tuppurainen M, et al. Dietary protein intake is associated with better physical function and muscle strength among elderly women. The British journal of nutrition. 2016:1-11.

126.   Aleman-Mateo H, Carreon VR, Macias L, Astiazaran-Garcia H, Gallegos-Aguilar AC, Enriquez JR. Nutrient-rich dairy proteins improve appendicular skeletal muscle mass and physical performance, and attenuate the loss of muscle strength in older men and women subjects: a single-blind randomized clinical trial. Clinical interventions in aging. 2014;9:1517-25.

127.   Zhu K, Kerr DA, Meng X, Devine A, Solah V, Binns CW, et al. Two-Year Whey Protein Supplementation Did Not Enhance Muscle Mass and Physical Function in Well-Nourished Healthy Older Postmenopausal Women. The Journal of nutrition. 2015;145(11):2520-6.

128.   Houston DK, Nicklas BJ, Ding J, Harris TB, Tylavsky FA, Newman AB, et al. Dietary protein intake is associated with lean mass change in older, community-dwelling adults: the Health, Aging, and Body Composition (Health ABC) Study. The American journal of clinical nutrition. 2008;87(1):150-5.

129.   Bartali B, Frongillo EA, Bandinelli S, Lauretani F, Semba RD, Fried LP, et al. Low nutrient intake is an essential component of frailty in older persons. The journals of gerontology Series A, Biological sciences and medical sciences. 2006;61(6):589-93.

130.   Granic A, Mendonca N, Sayer AA, Hill TR, Davies K, Adamson A, et al. Low protein intake, muscle strength and physical performance in the very old: The Newcastle 85+ Study. Clinical nutrition (Edinburgh, Scotland). 2017.

131.   Ottestad I, Lovstad AT, Gjevestad GO, Hamarsland H, Saltyte Benth J, Andersen LF, et al. Intake of a Protein-Enriched Milk and Effects on Muscle Mass and Strength. A 12-Week Randomized Placebo Controlled Trial among Community-Dwelling Older Adults. The journal of nutrition, health & aging. 2017;21(10):1160-9.

132.   Vaes AMM, Brouwer-Brolsma EM, Toussaint N, de Regt M, Tieland M, van Loon LJC, et al. The association between 25-hydroxyvitamin D concentration, physical performance and frailty status in older adults. European journal of nutrition. 2018.

133.   Bischoff-Ferrari HA, Dietrich T, Orav EJ, Hu FB, Zhang Y, Karlson EW, et al. Higher 25-hydroxyvitamin D concentrations are associated with better lower-extremity function in both active and inactive persons aged > or =60 y. The American journal of clinical nutrition. 2004;80(3):752-8.

134.   Visser M, Deeg DJ, Lips P. Low vitamin D and high parathyroid hormone levels as determinants of loss of muscle strength and muscle mass (sarcopenia): the Longitudinal Aging Study Amsterdam. The Journal of clinical endocrinology and metabolism. 2003;88(12):5766-72.

135.   Bischoff-Ferrari HA. Relevance of vitamin D in fall prevention. Geriatrie et psychologie neuropsychiatrie du vieillissement. 2017;15(1):E1-e7.

136.   Bischoff HA, Stahelin HB, Dick W, Akos R, Knecht M, Salis C, et al. Effects of vitamin D and calcium supplementation on falls: a randomized controlled trial. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research. 2003;18(2):343-51.

137.   Kojima G. Frailty as a Predictor of Future Falls Among Community-Dwelling Older People: A Systematic Review and Meta-Analysis. Journal of the American Medical Directors Association. 2015;16(12):1027-33.

138.   Bolland MJ, Grey A, Gamble GD, Reid IR. Vitamin D supplementation and falls: a trial sequential meta-analysis. The lancet Diabetes & endocrinology. 2014;2(7):573-80.

139.   Tricco AC, Thomas SM, Veroniki AA, Hamid JS, Cogo E, Strifler L, et al. Comparisons of Interventions for Preventing Falls in Older Adults: A Systematic Review and Meta-analysis. JAMA : the journal of the American Medical Association. 2017;318(17):1687-99.

140.   Annweiler C, Schott AM, Berrut G, Fantino B, Beauchet O. Vitamin D-related changes in physical performance: a systematic review. Journal of Nutrition, Health & Aging. 2009;13(10):893-8.

141.   Muir SW, Montero-Odasso M. Effect of vitamin D supplementation on muscle strength, gait and balance in older adults: a systematic review and meta-analysis. Journal of the American Geriatrics Society. 2011;59(12):2291-300.

142.   Rejnmark L. Effects of vitamin d on muscle function and performance: a review of evidence from randomized controlled trials. Therapeutic advances in chronic disease. 2011;2(1):25-37.

143.   Stockton KA, Mengersen K, Paratz JD, Kandiah D, Bennell KL. Effect of vitamin D supplementation on muscle strength: a systematic review and meta-analysis. Osteoporosis International. 2011;22(3):859-71.

144.   Beaudart C, Buckinx F, Rabenda V, Gillain S, Cavalier E, Slomian J, et al. The effects of vitamin D on skeletal muscle strength, muscle mass, and muscle power: a systematic review and meta-analysis of randomized controlled trials. The Journal of clinical endocrinology and metabolism. 2014;99(11):4336-45.

145.   Latham NKK. Effects of vitamin D supplementation on strength, physical performance, and falls in older persons: a systematic review. Journal of the American Geriatrics Society. 2003;51(9):1219-26.

146.   Darling AL, Millward DJ, Torgerson DJ, Hewitt CE, Lanham-New SA. Dietary protein and bone health: a systematic review and meta-analysis. The American journal of clinical nutrition. 2009;90(6):1674-92.

147.   Genaro Pde S, Pinheiro Mde M, Szejnfeld VL, Martini LA. Dietary protein intake in elderly women: association with muscle and bone mass. Nutr Clin Pract. 2015;30(2):283-9.

148.   Tang BM, Eslick GD, Nowson C, Smith C, Bensoussan A. Use of calcium or calcium in combination with vitamin D supplementation to prevent fractures and

bone loss in people aged 50 years and older: a meta-analysis. Lancet. 2007;370(9588):657-66.

149.   Holvik K, Ahmed LA, Forsmo S, Gjesdal CG, Grimnes G, Samuelsen SO, et al. Low serum levels of 25-hydroxyvitamin D predict hip fracture in the elderly: a NOREPOS study. The Journal of clinical endocrinology and metabolism. 2013;98(8):3341-50.

150.   Booth SL, Tucker KL, Chen H, Hannan MT, Gagnon DR, Cupples LA, et al. Dietary vitamin K intakes are associated with hip fracture but not with bone mineral density in elderly men and women. The American journal of clinical nutrition. 2000;71(5):1201-8.

151.   Finnes TE, Lofthus CM, Meyer HE, Sogaard AJ, Tell GS, Apalset EM, et al. A combination of low serum concentrations of vitamins K and D is associated with increased risk of hip fractures in elderly Norwegians: a NOREPOS study. Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA. 2015.

152.   Maggio M, Artoni A, Lauretani F, Borghi L, Nouvenne A, Valenti G, et al. The impact of omega-3 fatty acids on osteoporosis. Curr Pharm Des. 2009;15(36):4157-64.

153.   Benetou V, Orfanos P, Pettersson-Kymmer U, Bergstrom U, Svensson O, Johansson I, et al. Mediterranean diet and incidence of hip fractures in a European cohort. Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA. 2013;24(5):1587-98.

154.   Shams-White MM, Chung M, Du M, Fu Z, Insogna KL, Karlsen MC, et al. Dietary protein and bone health: a systematic review and meta-analysis from the National Osteoporosis Foundation. The American journal of clinical nutrition. 2017.

155.   Mangano KM, Sahni S, Kiel DP, Tucker KL, Dufour AB, Hannan MT. Bone Mineral Density and Protein-Derived Food Clusters from the Framingham Offspring Study. J Acad Nutr Diet. 2015;115(10):1605-13 e1.

156.   Langsetmo L, Barr SI, Berger C, Kreiger N, Rahme E, Adachi JD, et al. Associations of protein intake and protein source with bone mineral density and fracture risk: A population-based cohort study. The journal of nutrition, health & aging. 2015;19(8):861-8.

157.   Shams-White MM, Chung M, Fu Z, Insogna KL, Karlsen MC, LeBoff MS, et al. Animal versus plant protein and adult bone health: A systematic review and meta-analysis from the National Osteoporosis Foundation. PloS one. 2018;13(2):e0192459.

158.   Wallace TC, Frankenfeld CL. Dietary Protein Intake above the Current RDA and Bone Health: A Systematic Review and Meta-Analysis. Journal of the American College of Nutrition. 2017;36(6):481-96.

159.   Fung TT, Meyer HE, Willett WC, Feskanich D. Protein intake and risk of hip fractures in postmenopausal women and men age 50 and older. Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA. 2017;28(4):1401-11.

160.   Dawson-Hughes B, Harris SS. Calcium intake influences the association of protein intake with rates of bone loss in elderly men and women. The American journal of clinical nutrition. 2002;75(4):773-9.

161.   Sahni S, Cupples LA, McLean RR, Tucker KL, Broe KE, Kiel DP, et al. Protective effect of high protein and calcium intake on the risk of hip fracture in the Framingham offspring cohort. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research. 2010;25(12):2770-6.

162.   Grant AM, Avenell A, Campbell MK, McDonald AM, MacLennan GS, McPherson GC, et al. Oral vitamin D3 and calcium for secondary prevention of low-trauma fractures in elderly people (Randomised Evaluation of Calcium Or vitamin D, RECORD): a randomised placebo-controlled trial. Lancet. 2005;365(9471):1621-8.

163.   Jackson RD, LaCroix AZ, Gass M, Wallace RB, Robbins J, Lewis CE, et al. Calcium plus vitamin D supplementation and the risk of fractures. The New England journal of medicine. 2006;354(7):669-83.

164.   Weaver CM, Alexander DD, Boushey CJ, Dawson-Hughes B, Lappe JM, LeBoff MS, et al. Calcium plus vitamin D supplementation and risk of fractures: an

updated meta-analysis from the National Osteoporosis Foundation. Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA. 2016;27(1):367-76.

165.   Lips P, van Schoor NM. The effect of vitamin D on bone and osteoporosis. Best practice & research Clinical endocrinology & metabolism. 2011;25(4):585-91.

166.   Farina EK, Kiel DP, Roubenoff R, Schaefer EJ, Cupples LA, Tucker KL. Protective effects of fish intake and interactive effects of long-chain polyunsaturated fatty acid intakes on hip bone mineral density in older adults: the Framingham Osteoporosis Study. The American journal of clinical nutrition. 2011;93(5):1142-51.

167.   Virtanen JK, Mozaffarian D, Cauley JA, Mukamal KJ, Robbins J, Siscovick DS. Fish consumption, bone mineral density, and risk of hip fracture among older adults: the cardiovascular health study. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research. 2010;25(9):1972-9.

168.   Chen YM, Ho SC, Lam SS. Higher sea fish intake is associated with greater bone mass and lower osteoporosis risk in postmenopausal Chinese women. Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA. 2010;21(6):939-46.

169.   Zalloua PA, Hsu YH, Terwedow H, Zang T, Wu D, Tang G, et al. Impact of seafood and fruit consumption on bone mineral density. Maturitas. 2007;56(1):1-11.

170.   Calderon-Garcia JF, Moran JM, Roncero-Martin R, Rey-Sanchez P, Rodriguez-Velasco FJ, Pedrera-Zamorano JD. Dietary habits, nutrients and bone mass in Spanish premenopausal women: the contribution of fish to better bone health. Nutrients. 2012;5(1):10-22.

171.   Sahni S, Mangano KM, McLean RR, Hannan MT, Kiel DP. Dietary Approaches for Bone Health: Lessons from the Framingham Osteoporosis Study. Current osteoporosis reports. 2015;13(4):245-55.

172.   Perna S, Avanzato I, Nichetti M, D'Antona G, Negro M, Rondanelli M. Association between Dietary Patterns of Meat and Fish Consumption with Bone Mineral Density or Fracture Risk: A Systematic Literature. Nutrients. 2017;9(9).

173.   Poulsen RC, Moughan PJ, Kruger MC. Long-chain polyunsaturated fatty acids and the regulation of bone metabolism. Exp Biol Med (Maywood). 2007;232(10):1275-88.

174.   Lavado-Garcia J, Roncero-Martin R, Moran JM, Pedrera-Canal M, Aliaga I, Leal-Hernandez O, et al. Long-chain omega-3 polyunsaturated fatty acid dietary intake is positively associated with bone mineral density in normal and osteopenic Spanish women. PloS one. 2018;13(1):e0190539.

175.   Salari P, Rezaie A, Larijani B, Abdollahi M. A systematic review of the impact of n-3 fatty acids in bone health and osteoporosis. Med Sci Monit. 2008;14(3):RA37-44.

176.   Orchard TS, Pan X, Cheek F, Ing SW, Jackson RD. A systematic review of omega-3 fatty acids and osteoporosis. The British journal of nutrition. 2012;107 Suppl 2:S253-60.

177.   Fan F, Xue WQ, Wu BH, He MG, Xie HL, Ouyang WF, et al. Higher fish intake is associated with a lower risk of hip fractures in Chinese men and women: a matched case-control study. PloS one. 2013;8(2):e56849.

178.   Farina EK, Kiel DP, Roubenoff R, Schaefer EJ, Cupples LA, Tucker KL. Dietary intakes of arachidonic acid and alpha-linolenic acid are associated with reduced risk of hip fracture in older adults. The Journal of nutrition. 2011;141(6):1146-53.

179.   Nematy M, Hickson M, Brynes AE, Ruxton CH, Frost GS. Vulnerable patients with a fractured neck of femur: nutritional status and support in hospital. J Hum Nutr Diet. 2006;19(3):209-18.

180.   Calvani R, Martone AM, Marzetti E, Onder G, Savera G, Lorenzi M, et al. Pre-hospital dietary intake correlates with muscle mass at the time of fracture in older hip-fractured patients. Frontiers in aging neuroscience. 2014;6:269.

181.   van Wissen J, van Stijn MF, Doodeman HJ, Houdijk AP. Mini Nutritional Assessment and Mortality after Hip Fracture Surgery in the Elderly. The journal of nutrition, health & aging. 2016;20(9):964-8.

182.   Lumbers M, New SA, Gibson S, Murphy MC. Nutritional status in elderly female hip fracture patients: comparison with an age-matched home living group attending day centres. The British journal of nutrition. 2001;85(6):733-40.

183.   Eneroth M, Olsson UB, Thorngren KG. Insufficient fluid and energy intake in hospitalised patients with hip fracture. A prospective randomised study of 80 patients. Clinical nutrition (Edinburgh, Scotland). 2005;24(2):297-303.

184.   Paillaud E, Bories PN, Le Parco JC, Campillo B. Nutritional status and energy expenditure in elderly patients with recent hip fracture during a 2-month follow-up. The British journal of nutrition. 2000;83(2):97-103.

185.   Reider L, Hawkes W, Hebel JR, D'Adamo C, Magaziner J, Miller R, et al. The association between body mass index, weight loss and physical function in the year following a hip fracture. The journal of nutrition, health & aging. 2013;17(1):91-5.

186.   Wehren LE, Hawkes WG, Hebel JR, Orwig DL, Magaziner J. Bone mineral density, soft tissue body composition, strength, and functioning after hip fracture. The journals of gerontology Series A, Biological sciences and medical sciences. 2005;60(1):80-4.

187.   Visser M, Goodpaster BH, Kritchevsky SB, Newman AB, Nevitt M, Rubin SM, et al. Muscle mass, muscle strength, and muscle fat infiltration as predictors of incident mobility limitations in well-functioning older persons. The journals of gerontology Series A, Biological sciences and medical sciences. 2005;60(3):324-33.

188.   D'Adamo CR, Hawkes WG, Miller RR, Jones M, Hochberg M, Yu-Yahiro J, et al. Short-term changes in body composition after surgical repair of hip fracture. Age and ageing. 2014;43(2):275-80.

189.   Kenny AM, Biskup B, Robbins B, Marcella G, Burleson JA. Effects of vitamin D supplementation on strength, physical function, and health perception in older, community-dwelling men. Journal of the American Geriatrics Society [Internet]. 2003; 51(12):[1762-7 pp.].

190.   Glendenning P, Zhu K, Inderjeeth C, Howat P, Lewis JR, Prince RL. Effects of three-monthly oral 150,000 IU cholecalciferol supplementation on falls, mobility, and muscle strength in older postmenopausal women: a randomized controlled trial. Journal of Bone & Mineral Research. 2012;27(1):170-6.

191.   Grady D, Halloran B, Cummings S, Leveille S, Wells L, Black D, et al. 1,25-Dihydroxyvitamin D3 and muscle strength in the elderly: a randomized controlled trial. The Journal of clinical endocrinology and metabolism. 1991;73(5):1111-7.

192.   Podsiadlo D, Richardson S. The timed "Up & Go": a test of basic functional mobility for frail elderly persons. Journal of the American Geriatrics Society. 1991;39(2):142-8.

193.   Refsum H, Nurk E, Smith AD, Ueland PM, Gjesdal CG, Bjelland I, et al. The Hordaland Homocysteine Study: a community-based study of homocysteine, its determinants, and associations with disease. The Journal of nutrition. 2006;136(6 Suppl):1731S-40S.

194.   Gjesdal CG. Epidemiologic studies of osteoporosis in Hordaland. Bergen, Norway: University of Bergen; 2007.

195.   Gjesdal CG, Aanderud SJ, Haga HJ, Brun JG, Tell GS. Femoral and whole-body bone mineral density in middle-aged and older Norwegian men and women: suitability of the reference values. Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA. 2004;15(7):525-34.

196.   Gjesdal CG, Vollset SE, Ueland PM, Refsum H, Meyer HE, Tell GS. Plasma homocysteine, folate, and vitamin B 12 and the risk of hip fracture: the hordaland homocysteine study. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research. 2007;22(5):747-56.

197.   Apalset EM, Gjesdal CG, Eide GE, Tell GS. Intake of vitamin K1 and K2 and risk of hip fractures: The Hordaland Health Study. Bone. 2011;49(5):990-5.

198.   Blanton CA, Moshfegh AJ, Baer DJ, Kretsch MJ. The USDA Automated Multiple-Pass Method accurately estimates group total energy and nutrient intake. The Journal of nutrition. 2006;136(10):2594-9.

199.   Kyle UG, Bosaeus I, De Lorenzo AD, Deurenberg P, Elia M, Gomez JM, et al. Bioelectrical impedance analysis--part I: review of principles and methods. Clinical nutrition (Edinburgh, Scotland). 2004;23(5):1226-43.

200.   Tengvall M, Ellegard L, Malmros V, Bosaeus N, Lissner L, Bosaeus I. Body composition in the elderly: reference values and bioelectrical impedance spectroscopy to predict total body skeletal muscle mass. Clinical nutrition (Edinburgh, Scotland). 2009;28(1):52-8.

201.   Steihaug OM, Gjesdal CG, Bogen AH, Ranhoff AH. Identifying low muscle mass in patients with hip fracture: validation of bioelectrical impendance analysis and anthropometry compared to dual energy x-ray absorptiometry. The journal of nutrition, health & aging. 2015.

202.   Kyle UG, Genton L, Hans D, Pichard C. Validation of a bioelectrical impedance analysis equation to predict appendicular skeletal muscle mass (ASMM). Clinical nutrition (Edinburgh, Scotland). 2003;22(6):537-43.

203.   Parker MJ, Palmer CR. A new mobility score for predicting mortality after hip fracture. The Journal of bone and joint surgery British volume. 1993;75(5):797-8.

204.   McGilton KS, Chu CH, Naglie G, van Wyk PM, Stewart S, Davis AM. Factors Influencing Outcomes of Older Adults After Undergoing Rehabilitation for Hip Fracture. Journal of the American Geriatrics Society. 2016;64(8):1601-9.

205.   Pedersen TJ, Lauritsen JM. Routine functional assessment for hip fracture patients. Acta orthopaedica. 2016;87(4):374-9.

206.   Review Manager (RevMan). In: Center TNC, editor. 5.3 ed. Copenhagen: The Cochrane Collaboration; 2014.

207.   Higgins JPT, Green S. Cochrane Handbook for Systematic Reviews of Interventions. Version 5.1.0 ed: The Cochrane Collaboration; 2011 March 2011.

208.   Wood AD, Secombes KR, Thies F, Aucott LS, Black AJ, Reid DM, et al. A parallel group double-blind RCT of vitamin D_3 assessing physical function: Is the biochemical response to treatment affected by overweight and obesity? Osteoporosis International. 2014;25(1):305-15.

209.   Song F, Sheldon TA, Sutton AJ, Abrams KR, Jones DR. Methods for exploring heterogeneity in meta-analysis. Evaluation & the health professions. 2001;24(2):126-51.

210.   Yusuf S, Wittes J, Probstfield J, Tyroler HA. Analysis and interpretation of treatment effects in subgroups of patients in randomized clinical trials. JAMA : the journal of the American Medical Association. 1991;266(1):93-8.

211.   SRNT Subcommittee on Biochemical Verification. Biochemical verification of tobacco use and cessation. Nicotine Tob Res. 2002;4(2):149-59.

212.   Bischoff-Ferrari HA, Dawson-Hughes B, Orav EJ, Staehelin HB, Meyer OW, Theiler R, et al. Monthly High-Dose Vitamin D Treatment for the Prevention of Functional Decline: A Randomized Clinical Trial. JAMA Intern Med. 2016:1-10.

213.   Setiati S, Anugrahini, Fransiska JE, Tamin TZ, Istanti R. Combination of alfacalcidol and calcium improved handgrip strength and mobility among Indonesian older women: A randomized controlled trial. Geriatr Gerontol Int. 2018;18(3):434-40.

214.   Kondrup J, Allison SP, Elia M, Vellas B, Plauth M. ESPEN guidelines for nutrition screening 2002. Clinical nutrition (Edinburgh, Scotland). 2003;22(4):415-21.

215.   Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. BMJ (Clinical research ed). 1997;315(7109):629-34.

216.   Mijnarends DM, Meijers JM, Halfens RJ, ter Borg S, Luiking YC, Verlaan S, et al. Validity and reliability of tools to measure muscle mass, strength, and physical performance in community-dwelling older people: a systematic review. Journal of the American Medical Directors Association. 2013;14(3):170-8.

217.   Andersen LF, Solvoll K, Johansson LR, Salminen I, Aro A, Drevon CA. Evaluation of a food frequency questionnaire with weighed records, fatty acids, and alpha-tocopherol in adipose tissue and serum. Am J Epidemiol. 1999;150(1):75-87.

218.   Andersen LF, Solvoll K, Drevon CA. Very-long-chain n-3 fatty acids as biomarkers for intake of fish and n-3 fatty acid concentrates. The American journal of clinical nutrition. 1996;64(3):305-11.

219. Lofthus CM, Osnes EK, Falch JA, Kaastad TS, Kristiansen IS, Nordsletten L, et al. Epidemiology of hip fractures in Oslo, Norway. Bone. 2001;29(5):413-8.

220. Newman AB. An overview of the design, implementation, and analyses of longitudinal studies on aging. Journal of the American Geriatrics Society. 2010;58 Suppl 2:S287-91.

221. Karlsson T, Rosendahl-Riise H, Dierkes J, Drevon CA, Tell GS, Nygard O. Associations between fish intake and the metabolic syndrome and its components among middle-aged men and women: the Hordaland Health Study. Food & nutrition research. 2017;61(1):1347479.

222. Haugsgjerd TR, Dierkes J, Vollset SE, Vinknes KJ, Nygard OK, Seifert R, et al. Association between Weight Change and Mortality in Community Living Older People Followed for Up to 14 Years. The Hordaland Health Study (HUSK). The journal of nutrition, health & aging. 2017;21(8):909-17.

223. Riboli E, Hunt KJ, Slimani N, Ferrari P, Norat T, Fahey M, et al. European Prospective Investigation into Cancer and Nutrition (EPIC): study populations and data collection. Public Health Nutr. 2002;5(6b):1113-24.

224. Krokstad S, Langhammer A, Hveem K, Holmen TL, Midthjell K, Stene TR, et al. Cohort Profile: the HUNT Study, Norway. Int J Epidemiol. 2013;42(4):968-77.

225. Ranhoff AH, Holvik K, Martinsen MI, Domaas K, Solheim LF. Older hip fracture patients: three groups with different needs. BMC geriatrics. 2010;10:65.

226. Karlsson M, Nilsson JA, Sernbo I, Redlund-Johnell I, Johnell O, Obrant KJ. Changes of bone mineral mass and soft tissue composition after hip fracture. Bone. 1996;18(1):19-22.

227. Beaton GH, Burema J, Ritenbaugh C. Errors in the interpretation of dietary assessments. The American journal of clinical nutrition. 1997;65(4 Suppl):1100s-7s.

228. Subar AF, Freedman LS, Tooze JA, Kirkpatrick SI, Boushey C, Neuhouser ML, et al. Addressing Current Criticism Regarding the Value of Self-Report Dietary Data. The Journal of nutrition. 2015;145(12):2639-45.

229. Andersen LF, Nes M, Lillegaard IT, Sandstad B, Bjorneboe GE, Drevon CA. Evaluation of a quantitative food frequency questionnaire used in a group of Norwegian adolescents. European journal of clinical nutrition. 1995;49(8):543-54.

230.   Nes M, Frost Andersen L, Solvoll K, Sandstad B, Hustvedt BE, Lovo A, et al. Accuracy of a quantitative food frequency questionnaire applied in elderly Norwegian women. European journal of clinical nutrition. 1992;46(11):809-21.

231.   Hunter DJ, Rimm EB, Sacks FM, Stampfer MJ, Colditz GA, Litin LB, et al. Comparison of measures of fatty acid intake by subcutaneous fat aspirate, food frequency questionnaire, and diet records in a free-living population of US men. Am J Epidemiol. 1992;135(4):418-27.

232.   Baylin A, Kabagambe EK, Siles X, Campos H. Adipose tissue biomarkers of fatty acid intake. The American journal of clinical nutrition. 2002;76(4):750-7.

233.   Brunner E, Stallone D, Juneja M, Bingham S, Marmot M. Dietary assessment in Whitehall II: comparison of 7 d diet diary and food-frequency questionnaire and validity against biomarkers. The British journal of nutrition. 2001;86(3):405-14.

234.   Willett WC, Howe GR, Kushi LH. Adjustment for total energy intake in epidemiologic studies. The American journal of clinical nutrition. 1997;65(4 Suppl):1220S-8S; discussion 9S-31S.

235.   Gibson RS. Principles Of Nutritional Assessment: Oxford University Press; 2005.

236.   Kipnis V, Subar AF, Midthune D, Freedman LS, Ballard-Barbash R, Troiano RP, et al. Structure of dietary measurement error: results of the OPEN biomarker study. Am J Epidemiol. 2003;158(1):14-21; discussion 2-6.

237.   Conway JM, Ingwersen LA, Moshfegh AJ. Accuracy of dietary recall using the USDA five-step multiple-pass method in men: an observational validation study. Journal of the American Dietetic Association. 2004;104(4):595-603.

238.   Souverein OW, Dekkers AL, Geelen A, Haubrock J, de Vries JH, Ocke MC, et al. Comparing four methods to estimate usual intake distributions. European journal of clinical nutrition. 2011;65 Suppl 1:S92-101.

239.   Johansson G, Wikman A, Ahren AM, Hallmans G, Johansson I. Underreporting of energy intake in repeated 24-hour recalls related to gender, age, weight status, day of interview, educational level, reported food intake, smoking habits and area of living. Public Health Nutr. 2001;4(4):919-27.

240.   Robinson SM, Reginster JY, Rizzoli R, Shaw SC, Kanis JA, Bautmans I, et al. Does nutrition play a role in the prevention and management of sarcopenia? Clinical nutrition (Edinburgh, Scotland). 2017.

241.   Reid IR, Bolland MJ, Grey A. Effects of vitamin D supplements on bone mineral density: a systematic review and meta-analysis. Lancet. 2014;383(9912):146-55.

242.   Buoite Stella A, Gortan Cappellari G, Barazzoni R, Zanetti M. Update on the Impact of Omega 3 Fatty Acids on Inflammation, Insulin Resistance and Sarcopenia: A Review. International journal of molecular sciences. 2018;19(1).

243.   Smith GI, Atherton P, Reeds DN, Mohammed BS, Rankin D, Rennie MJ, et al. Dietary omega-3 fatty acid supplementation increases the rate of muscle protein synthesis in older adults: a randomized controlled trial. The American journal of clinical nutrition. 2011;93(2):402-12.

244.   Smith GI, Julliand S, Reeds DN, Sinacore DR, Klein S, Mittendorfer B. Fish oil-derived n-3 PUFA therapy increases muscle mass and function in healthy older adults. The American journal of clinical nutrition. 2015;102(1):115-22.

245.   Krzyminska-Siemaszko R, Czepulis N, Lewandowicz M, Zasadzka E, Suwalska A, Witowski J, et al. The Effect of a 12-Week Omega-3 Supplementation on Body Composition, Muscle Strength and Physical Performance in Elderly Individuals with Decreased Muscle Mass. International journal of environmental research and public health. 2015;12(9):10558-74.

246.   Di Monaco M, Vallero F, Di Monaco R, Tappero R. Prevalence of sarcopenia and its association with osteoporosis in 313 older women following a hip fracture. Archives of gerontology and geriatrics. 2011;52(1):71-4.

247.   Janssen I. Influence of sarcopenia on the development of physical disability: the Cardiovascular Health Study. Journal of the American Geriatrics Society. 2006;54(1):56-62.

248.   Van Ancum JM, Pijnappels M, Jonkman NH, Scheerman K, Verlaan S, Meskers CGM, et al. Muscle mass and muscle strength are associated with pre- and post-hospitalization falls in older male inpatients: a longitudinal cohort study. BMC geriatrics. 2018;18(1):116.

249.   Huo YR, Suriyaarachchi P, Gomez F, Curcio CL, Boersma D, Gunawardene P, et al. Comprehensive nutritional status in sarco-osteoporotic older fallers. The journal of nutrition, health & aging. 2015;19(4):474-80.

250.   Bruyere O, Cavalier E, Reginster JY. Vitamin D and osteosarcopenia: an update from epidemiological studies. Current opinion in clinical nutrition and metabolic care. 2017;20(6):498-503.

251.   Steihaug OM, Gjesdal CG, Bogen B, Kristoffersen MH, Lien G, Ranhoff AH. Sarcopenia in patients with hip fracture: A multicenter cross-sectional study. PloS one. 2017;12(9):e0184780.

252.   Di Monaco M, Castiglioni C, De Toma E, Gardin L, Giordano S, Di Monaco R, et al. Presarcopenia and sarcopenia in hip-fracture women: prevalence and association with ability to function in activities of daily living. Aging clinical and experimental research. 2015;27(4):465-72.

253.   Gonzalez-Montalvo JI, Alarcon T, Gotor P, Queipo R, Velasco R, Hoyos R, et al. Prevalence of sarcopenia in acute hip fracture patients and its influence on short-term clinical outcome. Geriatr Gerontol Int. 2016;16(9):1021-7.

254.   Weaver CM, McCabe LD, McCabe GP, Novotny R, Van Loan M, Going S, et al. Bone mineral and predictors of bone mass in white, Hispanic, and Asian early pubertal girls. Calcified tissue international. 2007;81(5):352-63.

255.   Bhudhikanok GS, Wang MC, Eckert K, Matkin C, Marcus R, Bachrach LK. Differences in bone mineral in young Asian and Caucasian Americans may reflect differences in bone size. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research. 1996;11(10):1545-56.

256.   Seeman E, Hopper JL, Bach LA, Cooper ME, Parkinson E, McKay J, et al. Reduced bone mass in daughters of women with osteoporosis. The New England journal of medicine. 1989;320(9):554-8.

257.   Soroko SB, Barrett-Connor E, Edelstein SL, Kritz-Silverstein D. Family history of osteoporosis and bone mineral density at the axial skeleton: the Rancho Bernardo Study. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research. 1994;9(6):761-9.

258.   Estrada K, Styrkarsdottir U, Evangelou E, Hsu YH, Duncan EL, Ntzani EE, et al. Genome-wide meta-analysis identifies 56 bone mineral density loci and reveals 14 loci associated with risk of fracture. Nature genetics. 2012;44(5):491-501.

259.   Kanis JA, Johnell O, Oden A, Johansson H, De Laet C, Eisman JA, et al. Smoking and fracture risk: a meta-analysis. Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA. 2005;16(2):155-62.

260.   Bielemann RM, Martinez-Mesa J, Gigante DP. Physical activity during life course and bone mass: a systematic review of methods and findings from cohort studies with young adults. BMC musculoskeletal disorders. 2013;14:77.

261.   Beaudart C, Dawson A, Shaw SC, Harvey NC, Kanis JA, Binkley N, et al. Nutrition and physical activity in the prevention and treatment of sarcopenia: systematic review. Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA. 2017;28(6):1817-33.

262.   Nikander R, Sievanen H, Heinonen A, Daly RM, Uusi-Rasi K, Kannus P. Targeted exercise against osteoporosis: A systematic review and meta-analysis for optimising bone strength throughout life. BMC medicine. 2010;8:47.

263.   Blundell JE, Gibbons C, Caudwell P, Finlayson G, Hopkins M. Appetite control and energy balance: impact of exercise. Obesity reviews : an official journal of the International Association for the Study of Obesity. 2015;16 Suppl 1:67-76.

264.   Nowson C, O'Connell S. Protein Requirements and Recommendations for Older People: A Review. Nutrients. 2015;7(8):6874-99.

REVIEW

Vitamin D supplementation and its influence on muscle strength and mobility in community-dwelling older persons: a systematic review and meta-analysis

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Abstract

Background: It has been suggested that vitamin D status or supplementation is important for maintaining or improving muscle strength and mobility in older adults. The study results, however, do not provide consistent results. We therefore aimed to summarise the available evidence systematically, including only studies conducted in community-dwelling older persons.

Methods: A systematic search of the literature was performed in April of 2016. The systematic review includes studies that used vitamin D with or without calcium supplementation as the exposure variable and various measurements of muscle strength and mobility. The meta-analysis was limited to studies using hand grip strength (HGS) and timed-up-and-go test as the outcome variables.

Results: A total of 15 studies out of 2408 articles from the literature search were included in the systematic review, providing 2866 participants above the age of 65 years. In the majority of studies, no improvement in muscle strength and mobility was observed after administration of vitamin D with or without calcium supplements. In the meta-analysis, we observed a non-significant change in HGS [+0.2 kg (95% confidence interval = −0.25 to 0.7 kg; seven studies)] and a small, significant increase in the timed-up-and-go test [0.3 s (95% confidence interval = 0.1 to 0.5 s; five studies)] after vitamin D supplementation. The meta-analyses showed a high degree of heterogeneity between the studies.

Conclusions: In conclusion, we observed no improvement in muscle strength after the administration of vitamin D with or without calcium supplements. We did find a small but significant deterioration of mobility. However, this is based on a limited number of studies and participants.

Introduction

Among other changes in body composition and functions, ageing involves a decrease in skeletal muscle mass and strength, and consequently also in mobility [1]. This loss of muscle mass and strength is called sarcopenia and is associated with falls, fractures, immobilisation and mortality. Sarcopenia has an estimated prevalence of 5–13% in 60–70 year olds and 11–50% in persons older than 80 years [2]. These numbers demonstrate that declining muscle mass and strength are significant and age-dependent problems in older persons. Early and continuous interventions may be key to limiting this decline and preserving both muscle mass and strength. A number

of dietary measures (supplementation with protein, energy, or *n*-3 polyunsaturated fatty acids, micronutrient supplementations) [3–8] and exercise interventions [9,10] or their combinations have been tested [11–14]. Among these interventions, supplementation with vitamin D has been promoted as having positive effects in older persons with respect to the risk of falls and fractures [15,16]. Usually, meta-analyses investigating the effect of vitamin D on the risk of falling include studies using vitamin D either with or without calcium supplements. Therefore, it is impossible to conclude whether vitamin D supplementation would be effective on its own or not. This can be regarded as a serious limitation of previous randomised controlled trials (RCTs) and meta-analyses and can also lead to inconsistent conclusions [17]. In a newer meta-analysis investigating primarily the effect of vitamin D on hip fractures in older adults [18], however, it was concluded that vitamin D with calcium was effective in preventing fractures, although the effect of vitamin D without calcium was not significant. An increased risk of falls can be seen as a consequence of low muscle strength and mass [19]. It has been estimated that the risk of falls increases with age and the presence of frailty and falls are a common cause of fractures in old adults [20]. The incidence of falls is difficult to measure, and falls may also have many other causes. Direct measurements of muscle strength and mobility are therefore required to study the effect of vitamin D.

Vitamin D deficiency is widespread in adult and older populations [21], even in populations without other overt nutrient deficiencies [22]. Vitamin D supplementation is usually combined with calcium supplementation, aiming to ensure sufficient calcium from the diet during vitamin D supplementation [23,24]. Vitamin D may exert its influence on skeletal muscle cells by the presence of the vitamin D receptor, and may also be needed for optimal muscle function [21] and adequate protein synthesis. In observational studies, an adequate 25(OH)D concentration was associated with better musculoskeletal function and muscle strength [25,26]. However, the optimal level of 25(OH)D in older persons is still unknown, although it is suggested to be ≥65 nmol L^{-1} [27].

Older people represent a very heterogeneous group. In general, community-dwelling older persons are younger and in better health and a better functional state compared to institutionalised individuals, although a high prevalence of comorbidities of chronic diseases may be present. In particular, institutionalised older persons show higher degrees of frailty, are more dependent in activities of daily living, and have a higher prevalence of cognitive decline. It is therefore justified to distinguish between these two groups when analysing dietary measures aimed at reducing the decline in muscle strength and mobility,

although a number of studies did not make this distinction [28–33]. However, vitamin D supplementation for the prevention of loss of muscle strength and mobility has not been established in either group. In addition, studies vary in their design, the type and length of the intervention, and the outcomes because different measurements of muscle strength and mobility have been used, and there is no common protocol for these assessments.

The objective of this systematic review and meta-analysis was to investigate the effects of vitamin D supplementation (with or without calcium) in community-dwelling older subjects on muscle strength and mobility, based on the results from RCTs.

Materials and methods

Data sources and search strategy

Relevant studies were identified by a systematic search of current literature using PubMed, Embase, Medline, Web of Science and the Cochrane Library, followed by a manual search of the extracted articles and existing reviews. The clinical trial registry 'ClinicalTrials.gov' was searched for unpublished trials. The search covered the period up to 13 April 2016. The search terms are presented in the Supporting information (Appendix S1).

The inclusion criteria are stated in Table 1. Differences in dosage, frequency, mode of delivery or the form of the vitamin D supplementation were not a cause for exclusion. It became apparent that only the outcomes comprising hand grip strength (HGS) and timed-up-and-go (TUG) were investigated in a sufficient number of studies to perform a quantitative meta-analysis, whereas other outcomes of muscle strength and mobility were only included in the systematic review. Two of the authors (HRR, JD) screened the article titles and abstracts to identify studies that were suitable for inclusion. Ninety-four articles were read as full

Table 1 Overview of the inclusion criteria for the present systematic review and meta-analysis

Design	Randomised controlled trials
Participants	Older persons >65 years of age
	Humans
	Community-dwelling
Intervention	Vitamin D supplementation – all forms and all doses, with or without calcium supplements or dietary advice
Comparator	Low dose of vitamin D or vitamin D metabolites or placebo, with or without calcium supplement
Outcome measures	
Systematic review	Measures of muscle strength and mobility
Meta-analysis	Hand grip strength (HGS)
	Timed-up-and-go test (TUG)

papers, and 15 studies were selected for systematic review (Fig. 1). These 15 studies also included one identified by searching clincialtrials.gov [34]. The 15 articles were evaluated by all authors. Three other studies identified from clinicaltrials.gov were either still ongoing or a study protocol, and were therefore not included.

The various outcomes used in these studies are described in the Supporting information (Appendix S2). The overall quality of the full articles was assessed using the CONSORT statement checklist for assessing quality of randomised clinical trials [35]. The CONSORT statements

are summarised in the Supporting information (Appendix S3).

Data collection

All relevant information was extracted from eligible studies and is available in Table 2. Any other information necessary for the review, such as potential covariates to the RCT (e.g. the season in which the RCT took place and any ultraviolet-B exposure), the dropout rate and compliance, was also noted when reported.

MEDLINE, PubMed, Embase, Web of Science and Cochrane Library were searched electronically.
2408 potential articles were selected. Headlines assessed for possible inclusion

2314 articles excluded:
2304 irrelevant content
10 not a study

94 articles extracted for full assessment and abstracts assessed for possible inclusion

79 studies excluded because they did not correspond to the inclusion criteria:
2 nursing home residents
5 exercise as a co-intervention
1 hormone therapy as co-intervention
1 protein supplement as an intervention
5 severely/chronically ill patients
7 outcome not muscle strength or mobility
3 irrelevant content
5 studies on adults
34 not an RCT
11 conference abstract
4 study protocol
1 Spanish article

15 studies included for **systematic review**

5 studies were excluded for meta-analysis because of other physical performance outcomes

10 studies included for the **meta-analysis**

7 for outcome hand grip strength (HGS)
5 for outcome timed-up-and-go (TUG)

Figure 1 Flow chart of the selection of studies on the effect of vitamin D supplementation with or without calcium supplements on muscle strength and mobility in the present systematic review and meta-analysis. RCT, randomised controlled trial.

Table 2 An overview of the studies included in the systematic review and meta-analysis

Study	Sample size (sex), n	Age (years)	Serum 25(OH)D status at baseline (nmol L⁻¹) [mean (SD)]	Method used for analysing 25(OH)D	Study duration	Study design	Comparator	Form and dosage of vitamin D	Calcium supplement (mg)	Physical performance measure
Bischoff-Ferrari (2012)[42]	20 (females)	C: 63.45 (7.78) I: 59.48 (6.27)	C: 35.45 (9.03)‡ I: 30.7 (10.2)‡	HPLC-MS/MS	4 months	Randomised, double-blinded trial	C: 800 IU D₃ day⁻¹ C: 5600 IU D₃ week⁻¹	I: 20 µg HyD day⁻¹ I: 140 µg HyD week⁻¹	Non	TUG (3 m) Knee extension Knee flexion strength Repeated sit-to-stand
Ceglia (2013)[34]	21 (females)	C: 80 (5) I: 76 (4)	C: 48.3 (8.8) I: 43.6 (10.3)	RIA (DiaSorin Inc., Stillwater, MN, USA)	4 months	Randomised, double-blind, placebo-controlled Single centre	Placebo	4000 IU (D₃) day⁻¹, oral	Non (dietary intake was assessed)	Knee extension SPPB (incl. 4 m TUG)
Dhesi (2004)[48]	139	C: 76.6 (6.1) I: 77.0 (6.3)	C: 25.0† (23.8–26.3) I: 26.8†(25.5–28.0)	IDS Gamma-B 25-OH immunoassay (IDS, Tyne & Wear, UK)	6 months	Randomised, double-blind, placebo-controlled	Placebo	600 000 IU (D₂) × 1 bolus inj.	Non	Quadriceps strength AFPT
Glendenning 2012[49]*	686 (females)	C: 76.5 (4) I: 76.9 (4)	C: 66.5 (27.1) I: 65.0 (17.8)	Liaison method (DiaSorin Inc.)	3, 6 and 9 months	Randomised, double-blind, placebo-controlled	Placebo	150 000 IU (D₃) every 3 months, oral	Advice: 1300 (supp/diet)	Grip strength (kg) TUG (3 m)
Grady (1991)[51]*	98	C: 78.9 (5.4) I: 79.4 (5.4)	C: 65.7 (51.4) I: 60.4 (35.3)	Microassay by Reinhart et al., 1984	1, 2, 4, 8, 12, 18 and 24 weeks	Randomised, double-blind, placebo-controlled	Placebo	0.5 µg (1,25-dihydroxyvitamin D₃) day⁻¹, oral	Non (dietary intake was assessed)	Grip strength (kg) Leg muscle strength
Janssen (2010)[53]*	70 (females)	C: 79.2 (6.7) I: 82.4 (4.9)	C: 34.3 (11.5) I: 32.6 (11.6)	NA	6 months	Randomised, double-blind, placebo-controlled	Placebo	400 IU (D₃) day⁻¹, oral	500	Knee extension Hand grip strength (kg) LEP TUG (4 m) Modified Cooper test
Kenny (2003)[52]*	65 (men)	76 (4)	C: 60 (18)‡ I: 65 (18)‡	Competitive protein binding (Endocrine Science Inc., Calabasas Hills, CA, USA)	6 months	Randomised, double-blind, placebo-controlled	Placebo	1000 IU (D₃) day⁻¹, oral	500	Leg extension strength Grip strength (kg) SPPB (incl. 3 m TUG)
Lagari (2013)[44]*	86	73.4 (6.4)	82.5 (25.0)‡	LC/MS/MS	6 months	Randomised, double-blinded trial	400 IU D₃ day⁻¹, oral	2000 IU (D₃) day⁻¹, oral	Calcium supplements was assessed	Grip strength (kg) Gait speed

Table 2. Continued

Study	Sample size (sex), n	Age (years)	Serum 25(OH)D status at baseline (nmol L⁻¹) [mean (SD)]	Method used for analysing 25(OH)D	Study duration	Study design	Comparator	Form and dosage of vitamin D	Calcium supplement (mg)	Physical performance measure
Lips (2010)[43]	593	C: 77.6 (6.6) I: 78.5 (6.2)	C: 35.3 (13.8)‡ I: 34.3 (11.0)‡	Reversed phase HPLC by Lensmeyer et al., 2006	16 weeks	Randomised, double-blind, placebo-controlled Multicentre	Placebo	8400 IU (D₃) week⁻¹, oral	500 for those with dietary intake <1000 mg	SPPB
Pfeifer (2009)[45]*	242	77 (4)	C: 54 (18) I: 55 (18)	RIA (Immunodiagnostic Systems, Boldon, UK)	12 and 20 months	Randomised, double-blind, placebo-controlled Multicentre	Placebo	800 IU (D₃) day⁻¹, oral	1000	Quadriceps strength (isometric leg extensor strength) TUG (3 m)
Pirotta (2015)[50]*	26	C: 71.5 (5.7) I: 66.1 (4.0)	C: 48.5 (11.1) I: 46.4 (11.4)	Liaison method (DiaSorin)	10 weeks	Randomised, double-blind, placebo-controlled	Placebo	2000 IU (D₃) day⁻¹, oral	Non	Knee extensor Stair climbing power FSST TUG (3 m)
Songpatanasilp (2009)[46]	72 (females)	70.60 (4.30)	69.98 (19.18)‡	RIA (DiaSorin)	12 weeks	Randomised placebo-controlled trial	Placebo	0.5 mg (20 000 IU) (alfacalcidiol) day⁻¹, oral	1500	Quadriceps strength (isokinetic dynamometer)
Wood (2014)[38]*	305 (females)	63.8 (2.2)	Normal: 34.3 (14.7) Overweight: 33.9 (14.3) Obese: 32.4 (16.3)	LC/MS/MS (Chromsystems, UK)	12 months (bimonthly study visits)	Randomised, double-blind, placebo-controlled	Placebo	I: 400 IU (D₃) day⁻¹, oral I: 1000 IU (D₃) day⁻¹, oral	Non	Grip strength (kg)
Xia (2009)[55]*	142 (females)	C: 70.4 (3.6) I: 70.4 (3.9)	NA	NA	6 and 12 months	Randomised, multicentre, open-label, placebo-controlled	125 IU (Calcitriol) day⁻¹, oral	125 IU + 0.25 µg (Calcitriol) day⁻¹, oral	600/600	Grip strength (kg) FTFFT
Zhu (2010)[47]*	302 (females)	C: 77.0 (4.8) I: 77.6 (4.2)	C: 44.3 (13.0)‡ I: 45.3 (12.5)‡	RIA (DiaSorin)	6 and 12 months	Randomised, double-blind, placebo-controlled	Placebo	1000 IU (D₂) day⁻¹, oral	1000	TUG (3 m) Lower limb muscle strength (ankle, knee, hip)

*Included in the meta-analysis.
†Geometric mean and 95% confidence interval.
‡Calculated to nmol L⁻¹ using coefficient of 2.5.

AFPT, aggregate functional performance time; C, control; FSST, the four square step test; FTFFT, Five-times-sit-to-stand-test; HPLC, high-performance liquid chromatography; HyD, 25-hydroxyvitamin D3; I, intervention; LC/MS/MS, liquid chromatography, tandem mass spectrometry; LEP, Leg extension power; NA, not available; RIA, radioimmunoassay; SPPB, Short Physical Performance Battery; TUG, timed-up-and-go.

Statistical analysis

Other outcomes than TUG and HGS were reviewed narratively as a result of the low number of studies evaluating these outcomes. Only for TUG and HGS did we find more than three studies for a quantitative meta-analysis. We used RevMan, version 5.3 (Cochrane collaboration) [36] for the analysis, with the outcome being represented by Forrest plots (Figs 2 and 3). Weighted mean differences for vitamin D versus placebo/control were calculated by subtracting the mean of the outcome of interest at the end of the study from the mean at baseline. Standard deviations (SDs) of the differences were calculated using a formula given in the *Cochrane Handbook* [37], applying correlation coefficients of 1.0 for the HGS and 0.8 for the TUG-test. Because significant heterogeneity was observed between studies with a fixed effect model, we finally applied a random effects model. Studies that included more than one intervention group [38] were treated by dividing the number of subjects in the control group by the number of comparisons at the same time as retaining the mean (SD) of the change according to the *Cochrane Handbook* [37].

Subgroup analysis was conducted with predefined study characteristics: baseline vitamin D status, oral administration of the supplement, daily dose of vitamin D, placebo group, supplementation with vitamin D_2 or D_3, and advice on calcium supplementation to explore possible reasons for the observed heterogeneity [39,40].

Results

Search results

As per the Quality of Reporting of Meta-analyses (QUOROM) [41] flow diagram (Fig. 1), 15 out of 2408 studies were included in the systematic review and 10 of the 15 were eligible for the meta-analysis.

Narrative review

Study characteristics

We included a total of 15 studies, with a total of 2866 participants aged 65 years and older. Two studies were included with an average age of the participants of 63.8 years (range 60–70 years) [38] and 61.5 years (range 50–70 years) [42], whereas the average age in the other studies included was between 70 and 80 years. The ratio of men to women was approximately 1 : 9 (229/2044), not including one study that did not specify the participants' sex ($n = 593$) [43]. The studies were conducted in
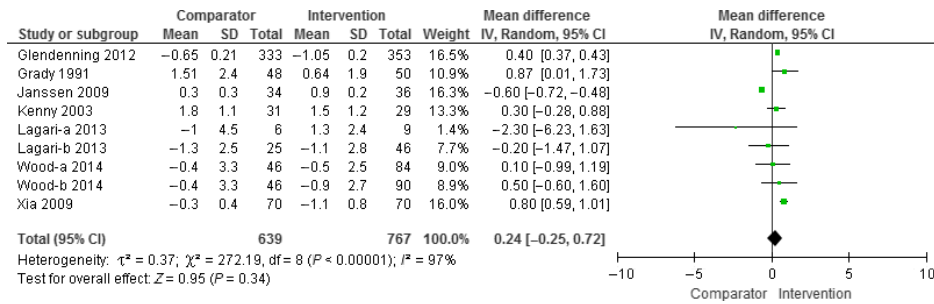
Figure 2 Results of the meta-analysis of the effect of vitamin D supplementation with or without calcium on hand grip strength (kg) ($n = 7$ studies). The results were obtained using a random effects model. One study reported results for men and women separately (Lagari a – men and Lagari b – women). For one study, we have divided the comparator group in two (Wood-a and Wood-b). CI, confidence interval.
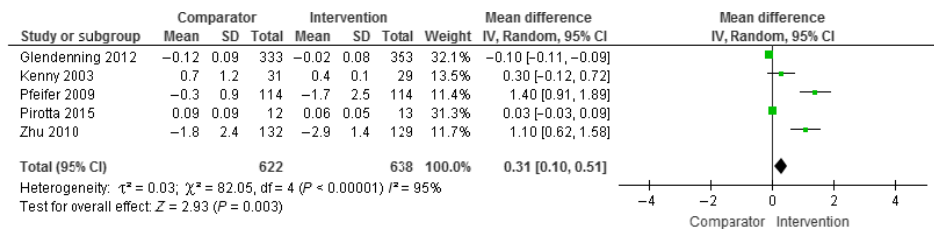
Figure 3 Results of the meta-analysis of the effect of vitamin D supplementation with or without calcium on timed-up-and-go (TUG) ($n = 5$ studies). The results were obtained using a random effects model. CI, confidence interval.

Australia, China, Thailand, USA, Canada and Europe (Germany, Austria, Netherlands, Switzerland, Scotland and the UK). One study was a multicentre study, with centres in North America, Mexico and Europe [43]. The participants were all community-dwelling older persons, who were generally in age-related good health, and a history of chronic conditions such as cardiovascular disease was usually not treated as an exclusion criterion. All studies excluded patients with acute diseases. In general, underlying diseases serving as exclusion criteria were not sufficiently described.

The vitamin D status was measured as the 25(OH)D concentration in 13 of the 15 studies, with chromatographic methods being used in four of them [38,42–44]. Other studies used radioimmunoassay (DiaSorin Inc., Stillwater, MN, USA) [34,45–47], IDS Gamma-B 25-OH immunoassay (IDS, Tyne & Wear, UK) [48], Liaison method (DiaSorin Inc.) [49,50] microassay as described per Reinhardt *et al.*, 1984 [51], competitive protein binding (Endocrine Science Inc., Calabasa Hills, CA, USA) [52]. One study did not report what method had been used [53].The mean baseline serum 25(OH)D concentration ranged between 25 and 82 nmol L^{-1} [54]. The average concentration exceeded the cut-off level of 50 nmol L^{-1} for defining a sufficient status, in six of the 15 studies [44–46,49,51,52] and, on average, was below that value in eight of them [34,38,42,43,47,48,50,53] and was not reported in one study [55].

All of the studies selected declared that they had a randomised parallel design. Three did not have a placebo group but used a low dose of vitamin D_3 (400 IU day^{-1}) as a control [44,55] or the recommended dose for elderly (800 IU day^{-1}) [42]. One study did not state whether it had been blinded or not [45]. One was a randomised, multicentre, open-label, placebo-controlled study [55]. The randomisation process was usually not sufficiently well described.

Seven of the 15 studies included calcium with vitamin D supplement or placebo [43,45–47,52,53,55]. Three studies assessed the intake of supplements or dietary calcium intake [34,44,51]. One study specified calcium supplementation or dietary intake of calcium of 1300 mg [47]. Two studies assessed the overall nutrient intake [38,50]. One study excluded participants using high-dose (>600 mg) calcium supplements [42]. One study did not consider calcium intake at all [48].

Both season and latitude can be important covariates as a result of internal vitamin D production by ultraviolet-B radiation [21]. The season of blood withdrawal was not stated in six of the 15 studies [34,42,50,53,55] and the geographic latitude was stated in three [38,45,47]. Other covariates of vitamin D status (body mass index, ethnicity, smoking) were usually not considered specifically.

The exception was one study that specifically investigated the effect modification of different body mass index groups (normal, overweight and obese) in participants with Caucasian ethnicity [38].

The dropout rate was given in 13 of the studies, and ranged from 0% to 22%. In one of these studies, the dropout rate was given in another publication [56]. In one study, the authors noted that the dropout rate was low, without further details [34]; in one study, the dropout rate was not reported at all [44].

Compliance was not mentioned in four of the 15 studies [43,46,52,55]. In eight of the studies, the compliance was reported to be better than 80% [38,42,44,47,49–51,53]. One study used bolus injections, so that 100% compliance may safely be assumed [48]. In one study, the compliance rate was 100% for the participants completing the study [49]. In one study, the authors stated that a daily compliance calendar had been kept but did not report on compliance [34].

Different metabolites of vitamin D were used, including vitamin D_2 [47,48], vitamin D_3 [34,38,43–45,49,50,52,53] 1,25-dihydroxyvitamin D_3 [51,55], alfacalcidiol [46] or 25(OH)D_3 [42] with various doses, administration routes and treatment periods, ranging from bolus injection of 600 000 IU of vitamin D_2 [48]; 1000 IU daily oral dose of vitamin D_2 [47]; oral vitamin D_3 in doses of 150 000 IU every 3 months [49]; weekly oral dose of 8400 IU vitamin D_3 [43]; and daily oral supplement in doses ranging from 400 to 4000 IU vitamin D_3 [34,38,44,45,50,52,53]. The studies that used 1,25-dihydroxyvitamin D used a daily oral dose of 1,25-dihydroxyvitamin D (0.5 μg) compared to placebo [51] or 125 IU (vitamin D_3) compared to 0.25 μg calcitriol 125 IU^{-1} [55]. One study included four groups comparing different doses of vitamin D3 (800 IU day^{-1} and 5600 IU week^{-1}) with 25(OH)D_3 (20 μg day^{-1} and 140 μg week^{-1}) [42].

An overview of the methodological quality of the studies is presented in the Supporting information (Appendix S3).

Study outcomes

Measurements of physical performance outcomes are not standardised, and various methods had been used in the clinical studies (see the Supporting information, Appendix S2). Four studies used complex outcome measurements such as the Short Physical Performance Battery (including TUG) [34,43,52] and the aggregate functional performance time [48].

Studies that used single outcome measurements included the knee extension test [34,42,50,53]. Three studies used quadriceps strength (using various protocols thus precluding a formal meta-analysis) [45,46,48],

HGS [38,44,49,51–53,55] and the 3-m TUG [42,45,47,49,50,52]. Other available physical outcome measures were the 4-m TUG, leg muscle strength, leg extension strength, gait speed, Five-Times-Sit-to-Stand-Test, leg extension power, modified Cooper test, stair climbing power, the four square step test, repeated sit-to-stand, knee flexion strength and lower limb muscle strength.

The authors of nine studies concluded that supplementation with vitamin D and/or calcium did not have any beneficial effect on mobility and/or muscle strength [34,38,43,44,48,49,51–53]. In six studies, they found an improvement in mobility and/or muscle strength [42,45–47,50,55]. One of the four studies that used a complex outcome measurement [48] reported a beneficial effect for the mobility outcome [48]. Three of the studies reporting an improvement in either measure only observed this in the subjects who had been weakest and slowest at baseline [47] or in those with pre-existing low levels of 25(OH)D3 [42,46]. In the case of one study [47], no subgroup analysis had been prespecified in the record of the trial registry (clinicaltrials.gov).

The meta-analysis

We performed meta-analyses for the outcomes HGS (kg) and TUG (s).

Hand grip strength

The meta-analysis included seven studies [38,44,49,51–53,55], with 767 participants treated with vitamin D and 639 participants treated with control (low-dose vitamin D or placebo). HGS was measured using various devices and protocols, giving an average HGS at baseline of between 3 and 23 kg. Applying a random effects model, we observed a nonsignificant improvement in HGS after vitamin D supplementation, amounting to 0.2 kg [95% confidence interval (CI) −0.3 to 0.7 kg]. The meta-analysis revealed significant heterogeneity between the studies ($I^2 = 97\%$), which was completely eliminated by omitting the three studies that included subjects with vitamin D deficiency [38,53,55]. After exclusion of these three studies, the effect on the HGS became significant (0.40, 95% CI = 0.37 to 0.43kg). Other sensitivity analyses (Table 1; see also Supporting information, Appendix S4: exclusion of studies using vitamin D_2, using bolus doses of vitamin D or inclusion of calcium supplements) did not diminish the heterogeneity between the studies and did not change the overall result of a marginal effect of vitamin D supplementation on HGS (Fig. 2).

Timed-up-and-go

The meta-analysis included five studies [45,47,49,50,52] with 638 participants treated with vitamin D and 622 participants treated with a control or placebo. The studies reported average TUG results ranging from 5 to 11 s. Applying a random effects model, we observed a significant mean increase of 0.3 s in the TUG (95% CI = 0.1 to 0.5 s) after vitamin D supplementation. Thus, the increase would mean a deterioration of the TUG result after vitamin D supplementation. The meta-analysis revealed significant heterogeneity between the studies ($I^2 = 95\%$) (Fig. 3). A sensitivity analysis excluding Zhu *et al.* [47] (who used vitamin D_2 as a supplement and included participants with an average 25(OH)D concentration lower than 50 nmol L^{-1}) lead to an insignificant overall estimate of 0.2 s (95% CI = −0.03 to 0.4s) but did not affect the heterogeneity (sensitivity analysis presented in Table 2; see also Supporting information, Appendix S4).

Discussion

The objective of this systematic review and meta-analysis was to investigate whether vitamin D supplementation (with or without calcium) in community-dwelling older persons can improve muscle strength and mobility. For the present review, 15 RCTs were included for revision, whereas 10 were suitable for the meta-analysis. Based on findings in nine of the studies, it was concluded that supplementation with vitamin D and/or calcium did not have any beneficial effect on mobility or muscle strength, or on both [34,38,43,44,48,49,51–53]. The main findings of the quantitative meta-analysis indicated that supplementation with vitamin D did not improve the HGS (based on seven studies) to any significant extent and even had a worsening effect on the TUG-test results (based on five studies). Therefore, vitamin D supplementation appears to be of limited value for the preservation of muscle strength and mobility in an older population.

Study population

The older population is heterogeneous in age and the related frailty, as well as with respect to the prevalence of chronic diseases and their treatment, and dependence in the activities of daily life. It can therefore be expected that studies in older persons in general will yield mixed results unless the population is defined more accurately according to the factors mentioned. We therefore limited the present meta-analysis to community-dwelling older persons in apparently age-related good health, although, in many cases, the health status had not been sufficiently well described.

Community-dwelling older persons are usually in much better health than those hospitalised or living in nursing homes. Targeting these subjects with an intervention

aimed at preserving muscle strength and mobility thus appears sensible. However, in concordance with our findings, studies on vitamin D supplementation in hospitalised older subjects [57,58] or residents in nursing homes [3,7] showed mixed results for the effects of vitamin D supplementation on muscle strength and mobility. Thus, convincing evidence that vitamin D supplementation may be a useful measure is lacking [14,28,59].

Most studies recruited only or predominantly women. Although, at present, there is little evidence that the dietary requirements for vitamin D are different in older men and older women [60], or that the effects of vitamin D on muscle strength are different in older men and women, there is clearly a lack of data on the effect of vitamin D supplementation in men.

Intervention

Vitamin D exists in two different forms (D_3 and D_2). In addition, the inactive form [25(OH)D] and the active form of the hormone [1,25(OH)2D], different routes of administration (oral or intravenous, daily/weekly or bolus supplementation), as well as various doses and various durations of supplementation, can be used. These aspects further complicate comparison of the studies and can introduce heterogeneity between the studies. High-dose bolus supplementation (either oral or intravenous) has the advantage of high compliance, especially in older subjects who already take a number of medicines on a daily basis. Doses of 300 000 IU are an established treatment for vitamin D deficiency and are regarded as safe. However, doses over 500 000 IU should be avoided because adverse effects of such high doses such as increased falls and fracture risk have been reported [61,62].

The studies using a low dose were included in the meta-analysis as a result of studies by Lagari *et al.* [63] and Chao *et al.* [64] stating that 400 IU was inadequate to increase the 25(OH)D concentrations to an acceptable level regardless of baseline 25(OH)D levels in older persons. Because of the high dose of vitamin D_3 used as a control group in the study by Bischoff-Ferrari *et al.* [42], we choose not include the study in our meta-analysis.

Outcomes

The functional improvement in the older persons has been measured using a range of measurements employing different protocols. Among these, the HGS has been shown to be a reliable parameter [65,66] for long-term health outcomes. There is, however, less evidence for the TUG test for long-term health outcomes.

We included only quantifiable outcomes in the present study but not falls or fractures that have been used as

measure of reduced muscle strength and as clinical outcomes in other studies [15,67]. However, determining falls may be difficult in community-dwelling older persons because it relies heavily on the subjects' recall and may thus reduce the reliability of this outcome.

We observed a small and nonsignificant improvement in HGS as a result of vitamin D supplementation in the meta-analysis, which was also characterised by a high degree of heterogeneity between studies. However, the magnitude of the effect may also indicate that other health measures, such as exercise and potentially supplementation with other nutrients, should be prioritised. In addition, the huge variation in baseline HGS measurements between studies further complicates the interpretation of the effects of high/increased vitamin D intake. The nonsignificant result may be regarded as contradicting observational studies in community-dwelling older persons because other studies have reported that a doubling of the 25(OH)D concentration from 50 to 100 nmol L^{-1} was associated with a higher HGS in men and in women, with increases of approximately 4.4 and 0.8 kg, respectively [68]. We observed a significant and stronger improvement of HGS and diminished heterogeneity after the exclusion of three studies with low 25 (OH)D concentrations at baseline [38,53,55]. Low vitamin D status may reflect a higher degree of frailty [69], and supplementation may therefore be too late to improve muscle strength in those with very low 25(OH)D levels, despite the correction of vitamin D deficiency as indicated by the serum levels.

Although the effect of vitamin D supplementation on the TUG test suggests a negative direction by increasing the time used for the test, this result should be taken with caution because the meta-analysis showed a high degree of heterogeneity that was not removed by excluding single studies (Table 2; see also Supporting information, Appendix S4). In addition, the overall magnitude of the effect was very small, suggesting that this change is clinically less meaningful. Overall, the small number of studies and the high degree of heterogeneity precludes any firm conclusions, although further investigations are certainly warranted. It would be interesting to determine whether interventions combined with exercise and/or other nutrients would improve the test outcome. This has already been shown by Bunout *et al.* [12], who used exercise and vitamin D supplements as interventions in vitamin D-deficient community-dwelling older persons and observed a positive effect on TUG.

The importance of calcium supplements should also be considered. Because of the concurrent administration of calcium in most and especially in the larger studies [43,45–47,49,52,53,55], it is impossible to determine any independent effect of either vitamin D or calcium. The use of

calcium supplements for purposes other than improvement of bone health has been strongly debated. It is also plausible to combine vitamin D supplements with calcium because vitamin D increases calcium absorption from the gut but, in the case of insufficient dietary calcium intake, this can also affect bone remodelling [70].

Comparison with previous systematic reviews and meta-analyses

The effect of vitamin D on physical performance has been summarised in systematic reviews [28,30,33] and in three meta-analyses [29,31,32]. These investigations are characterised by either including all age groups [30–32], by including older adults from different settings (community-dwelling and institutionalised, [14,28,29], different study designs [33] and investigating composite outcomes [32], thus making comparisons with our findings difficult. Stockton *et al.* [31] also reported a meta-analysis for HGS and, in line with our findings, reported no significant effect on HGS. The only other meta-analysis that reported TUG as an outcome reported a small, significant improvement of this test, based on three studies [29].

Thus, the overall results are difficult to compare, although they demonstrate the large number of tests used for the assessment of muscle strength, physical performance and mobility. A common test battery would make comparisons between studies much easier.

Strengths and limitations

The strengths of this review include the use of data from 15 RCTs, with approximately 2800 participants treated with vitamin D or a control, and the analysis of quantitative outcomes such as HGS and TUG, which have been shown to be related to other clinical outcomes in the older persons [71].

The main limitation of this review is the small number of studies available for the meta-analysis, mainly as a result of heterogeneity of the measurements used. Another limitation is the variation in study populations, with a wide range of comorbidities. More exact descriptions of the population under study are urgently needed to improve comparability of studies and to increase external validity. We also observed heterogeneity between studies that could not be resolved by subgroup analyses.

In conclusion, we observed no improvement in muscle strength after administration of vitamin D with or without calcium supplements. We did find a small but significant deterioration of mobility. This is, however, based on a limited number of studies and participants.

Transparency declaration

The lead author affirms that this manuscript is an honest, accurate and transparent account of the study being reported, that no important aspects of the study have been omitted and that any discrepancies from the study as planned (and registered with) have been explained. The reporting of this work is compliant with CONSORT[1]/STROBE[2]/PRISMA[3] guidelines.

References

1. Cruz-Jentoft AJ, Baeyens JP, Bauer JM *et al.* (2010) Sarcopenia: European consensus on definition and diagnosis: report of the European Working Group on Sarcopenia in Older People. *Age Ageing* **39**, 412–423.
2. Morley JE (2012) Sarcopenia in the elderly. *Fam Pract* **29** (Suppl 1), i44–i48.
3. Smedshaug GB, Pedersen JI & Meyer HE (2007) Can vitamin D supplementation improve grip strength in elderly nursing home residents? A double-blinded controlled trial. *Scand J Food Nutr* **51**, 74–78.
4. Bacon CJ, Gamble GD, Horne AM *et al.* (2009) High-dose oral vitamin D3 supplementation in the elderly. *Osteoporos Int* **20**, 1407–1415.
5. Gallagher JC (2004) The effects of calcitriol on falls and fractures and physical performance tests. *J Steroid Biochem Mol Biol* **89–90**, 497–501.
6. Malafarina V, Uriz-Otano F, Iniesta R *et al.* (2013) Effectiveness of nutritional supplementation on muscle

mass in treatment of sarcopenia in old age: a systematic review. *J Am Med Dir Assoc* **14**, 10–17.

7. Moreira-Pfrimer LD, Pedrosa MA, Teixeira L *et al.* (2009) Treatment of vitamin D deficiency increases lower limb muscle strength in institutionalized older people independently of regular physical activity: a randomized double-blind controlled trial. *Ann Nutr Metab* **54**, 291–300.

8. Aleman-Mateo H, Macias L, Esparza-Romero J *et al.* (2012) Physiological effects beyond the significant gain in muscle mass in sarcopenic elderly men: evidence from a randomized clinical trial using a protein-rich food. *Clin Interv Aging* **7**, 225–234.

9. Liu CK, Leng X, Hsu FC *et al.* (2014) The impact of sarcopenia on a physical activity intervention: the Lifestyle Interventions and Independence for Elders Pilot Study (LIFE-P). *J Nutr Health Aging* **18**, 59–64.

10. Scanlon TC, Fragala MS, Stout JR *et al.* (2014) Muscle architecture and strength: adaptations to short-term resistance training in older adults. *Muscle Nerve* **49**, 584–592.

11. Hara S, Kishimoto KN, Okuno H *et al.* (2013) Effects of alfacalcidol on back extensor strength gained through back extensor exercise in postmenopausal women with osteoporosis. *Am J Phys Med Rehabil* **92**, 101–110.

12. Bunout D, Barrera G, Leiva L *et al.* (2006) Effects of vitamin D supplementation and exercise training on physical performance in Chilean vitamin D deficient elderly subjects. *Exp Gerontol* **41**, 746–752.

13. Burd NA, Yang Y, Moore DR *et al.* (2012) Greater stimulation of myofibrillar protein synthesis with ingestion of whey protein isolate v. micellar casein at rest and after resistance exercise in elderly men. *Br J Nutr* **108**, 958–962.

14. Latham NK, Anderson CS, Lee A *et al.* (2003) A randomized, controlled trial of quadriceps resistance exercise and vitamin D in frail older people: the Frailty Interventions Trial in Elderly Subjects (FITNESS). *J Am Geriatr Soc* **51**, 291–299.

15. Bischoff-Ferrari HA, Dawson-Hughes B, Staehelin HB *et al.* (2009) Fall prevention with supplemental and active forms of vitamin D: a meta-analysis of randomised controlled trials. *BMJ* **339**, b3692.

16. Murad MH, Elamin KB, Abu Elnour NO *et al.* (2011) Clinical review: the effect of vitamin D on falls: a systematic review and meta-analysis. *J Clin Endocrinol Metab* **96**, 2997–3006.

17. Scragg R (2012) Do we need to take calcium with vitamin D supplements to prevent falls, fractures, and death? *Curr Opin Clin Nutr Metab Care* **15**, 614–624.

18. Avenell A, Mak Jenson CS & O'Connell D (2014) Vitamin D and vitamin D analogues for preventing fractures in post-menopausal women and older men. *Cochrane Database Syst Rev* **4**, CD000227.

19. Kojima G (2015) Frailty as a predictor of future falls among community-dwelling older people: a systematic review and meta-analysis. *J Am Med Dir Assoc* **16**, 1027–1033.

20. Deandrea, S, Lucenteforte, E, Bravi, F *et al.* (2010) Risk factors for falls in community-dwelling older people: a systematic review and meta-analysis. *Epidemiology* **21**, 658–668.

21. Holick MF (2007) Vitamin D deficiency. *N Engl J Med* **357**, 266–281.

22. Brouwer-Brolsma EM, Bischoff-Ferrari HA, Bouillon R *et al.* (2013) Vitamin D: do we get enough? A discussion between vitamin D experts in order to make a step towards the harmonisation of dietary reference intakes for vitamin D across Europe. *Osteoporos Int* **24**, 1567–1577.

23. Tang BM, Eslick GD, Nowson C *et al.* (2007) Use of calcium or calcium in combination with vitamin D supplementation to prevent fractures and bone loss in people aged 50 years and older: a meta-analysis. *Lancet* **370**, 657–666.

24. Avenell A, Mak JC & O'Connell D (2014) Vitamin D and vitamin D analogues for preventing fractures in post-menopausal women and older men. *Cochrane Database Syst Rev* **4**, CD000227.

25. Bischoff-Ferrari HA, Borchers M, Gudat F *et al.* (2004) Vitamin D receptor expression in human muscle tissue decreases with age. *J Bone Miner Res* **19**, 265–269.

26. Visser M, Deeg DJ & Lips P (2003) Low vitamin D and high parathyroid hormone levels as determinants of loss of muscle strength and muscle mass (sarcopenia): the Longitudinal Aging Study Amsterdam. *J Clin Endocrinol Metab* **88**, 5766–5772.

27. Dawson-Hughes B (2008) Serum 25-hydroxyvitamin D and functional outcomes in the elderly. *Am J Clin Nutr* **88**, 537s–540s.

28. Annweiler C, Schott AM, Berrut G *et al.* (2009) Vitamin D-related changes in physical performance: a systematic review. *J Nutr Health Aging* **13**, 893–898.

29. Muir SW & Montero-Odasso M (2011) Effect of vitamin D supplementation on muscle strength, gait and balance in older adults: a systematic review and meta-analysis. *J Am Geriatr Soc* **59**, 2291–2300.

30. Rejnmark L (2011) Effects of vitamin d on muscle function and performance: a review of evidence from randomized controlled trials. *Ther Adv Chronic Dis* **2**, 25–37.

31. Stockton KA, Mengersen K, Paratz JD *et al.* (2011) Effect of vitamin D supplementation on muscle strength: a systematic review and meta-analysis. *Osteoporos Int* **22**, 859–871.

32. Beaudart C, Buckinx F, Rabenda V *et al.* (2014) The effects of vitamin D on skeletal muscle strength, muscle mass, and muscle power: a systematic review and meta-analysis of randomized controlled trials. *J Clin Endocrinol Metab* **99**, 4336–4345.

33. Latham NKK (2003) Effects of vitamin D supplementation on strength, physical performance, and falls in older persons: a systematic review. *J Am Geriatr Soc* **51**, 1219–1226.

34. Ceglia L, Niramitmahapanya S, da Silva Morais M *et al.* (2013) A randomized study on the effect of vitamin D3 supplementation on skeletal muscle morphology and vitamin d receptor concentration in older women. *J Clin Endocrinol Metab* **98**, E1927–E1935.

35. Schulz KF, Altman DG & Moher D (2010) CONSORT 2010 statement: updated guidelines for reporting parallel group randomized trials. *Ann Intern Med* **152**, 726–732.

36. The Cochrane Collaboration (2014) *Review Manager (RevMan)*, 5.3 edn. [TNC Center, editor]. Copenhagen: The Cochrane Collaboration.

37. Higgins JPT & Green S (2011) *Cochrane Handbook for Systematic Reviews of Interventions*, Version 5.1.0. Available at: www.cochrane-handbook.org. The Cochrane Collaboration.

38. Wood AD, Secombes KR, Thies F *et al.* (2014) A parallel group double-blind RCT of vitamin D₃ assessing physical function: is the biochemical response to treatment affected by overweight and obesity? *Osteoporos Int* **25**, 305–315.

39. Song F, Sheldon TA, Sutton AJ *et al.* (2001) Methods for exploring heterogeneity in meta-analysis. *Eval Health Prof* **24**, 126–151.

40. Yusuf S, Wittes J, Probstfield J *et al.* (1991) Analysis and interpretation of treatment effects in subgroups of patients in randomized clinical trials. *JAMA* **266**, 93–98.

41. Moher D, Cook DJ, Eastwood S *et al.* (1999) Improving the quality of reports of meta-analyses of randomised controlled trials: the QUOROM statement. Quality of reporting of meta-analyses. *Lancet* **354**, 1896–1900.

42. Bischoff-Ferrari HA, Dawson-Hughes B, Stocklin E *et al.* (2012) Oral supplementation with 25(OH)D3 versus vitamin D3: effects on 25(OH)D levels, lower extremity function, blood pressure, and markers of innate immunity. *J Bone Miner Res* **27**, 160–169.

43. Lips P, Binkley N. Pfeifer M *et al.* (2010) Once-weekly dose of 8400 IU vitamin D(3) compared with placebo: effects on neuromuscular function and tolerability in older adults with vitamin D insufficiency. *Am J Clin Nutr* **91**, 985–991.

44. Lagari V, Gomez-Marin O & Levis S (2013) The role of vitamin D in improving physical performance in the elderly. *J Bone Miner Res* **28**, 2194–2201.

45. Pfeifer M, Begerow B, Minne HW *et al.* (2009) Effects of a long-term vitamin D and calcium supplementation on falls and parameters of muscle function in community-dwelling older individuals. *Osteoporos Int* **20**, 315–322.

46. Songpatanasilp T, Chailurkit LO, Nichachotsalid A *et al.* (2009) Combination of alfacalcidol with calcium can improve quadriceps muscle strength in elderly ambulatory Thai women who have hypovitaminosis D: a randomized controlled trial. *J Med Assoc Thai* **92**(Suppl 5), S30–S41.

47. Zhu K, Austin N, Devine A *et al.* (2010) A randomized controlled trial of the effects of vitamin D on muscle strength and mobility in older women with vitamin D insufficiency. *J Am Geriatr Soc* **58**, 2063–2068.

48. Dhesi JK, Jackson SH, Bearne LM *et al.* (2004) Vitamin D supplementation improves neuromuscular function in older people who fall. *Age Ageing* **33**, 589–595.

49. Glendenning P, Zhu K, Inderjeeth C *et al.* (2012) Effects of three-monthly oral 150,000 IU cholecalciferol supplementation on falls, mobility, and muscle strength in older postmenopausal women: a randomized controlled trial. *J Bone Miner Res* **27**, 170–176.

50. Pirotta S, Kidgell DJ & Daly RM (2015) Effects of vitamin D supplementation on neuroplasticity in older adults: a double-blinded, placebo-controlled randomised trial. *Osteoporos Int* **26**, 131–140.

51. Grady D, Halloran B, Cummings S *et al.* (1991) 1,25-Dihydroxyvitamin D3 and muscle strength in the elderly: a randomized controlled trial. *J Clin Endocrinol Metab* **73**, 1111–1117.

52. Kenny AM, Biskup B, Robbins B *et al.* (2003) Effects of vitamin D supplementation on strength, physical function, and health perception in older, community-dwelling men. *J Am Geriatr Soc* **51**, 1762–1767.

53. Janssen H, Samson MM & Verhaar HJJ (2010) Muscle strength and mobility in vitamin D-insufficient female geriatric patients: a randomized controlled trial on vitamin D and calcium supplementation. *Aging Clin Exp Res* **22**, 78–84.

54. Ross AC, Taylor CL & Yaktine AL (2011) *Dietary Reference Intakes for Calcium and Vitamin D.* Washington, DC: National Academies Press (US).

55. Xia WB, Zhang ZL, Wang HF *et al.* (2009) The efficacy and safety of calcitriol and/or Caltrate D in elderly Chinese women with low bone mass. *Acta Pharmacol Sin* **30**, 372–378.

56. Wood AD, Secombes KR, Thies F *et al.* (2012) Vitamin D3 supplementation has no effect on conventional cardiovascular risk factors: a parallel-group, double-blind, placebo-controlled RCT. *J Clin Endocrinol Metab* **97**, 3557–3568.

57. Boxer RS, Kenny AM, Schmotzer BJ *et al.* (2013) A randomized controlled trial of high dose vitamin d3 in patients with heart failure. *JACC Heart Fail* **1**, 84–90.

58. Sato Y, Iwamoto J, Kanoko T *et al.* (2005) Low-dose vitamin D prevents muscular atrophy and reduces falls and hip fractures in women after stroke: a randomized controlled trial. *Cerebrovasc Dis* **20**, 187–192.

59. Brunner RL, Cochrane B, Jackson RD *et al.* (2008) Calcium, vitamin D supplementation, and physical function in the Women's Health Initiative. *J Am Diet Assoc* **108**, 1472–1479.

60. Cashman KD, Wallace JM, Horigan G *et al.* (2009) Estimation of the dietary requirement for vitamin D in free-living adults >=64 y of age. *Am J Clin Nutr* **89**, 1366–1374.

61. Sanders KM, Stuart AL, Williamson EJ *et al.* (2010) Annual high-dose oral vitamin D and falls and fractures in

older women: a randomized controlled trial. *JAMA* **303**, 1815–1822.

62. Rossini M, Gatti D, Viapiana O *et al.* (2012) Short-term effects on bone turnover markers of a single high dose of oral vitamin D(3). *J Clin Endocrinol Metab* **97**, E622–E626.

63. Lagari VS, Gomez-Marin O & Levis S (2012) Differences in vitamin D3 dosing regimens in a geriatric community-dwelling population. *Endocr Pract* **18**, 847–854.

64. Chao YS, Brunel L, Faris P *et al.* (2013) The importance of dose, frequency and duration of vitamin D supplementation for plasma 25-hydroxyvitamin D. *Nutrients* **5**, 4067–4078.

65. Mijnarends DM, Meijers JM, Halfens RJ *et al.* (2013) Validity and reliability of tools to measure muscle mass, strength, and physical performance in community-dwelling older people: a systematic review. *J Am Med Dir Assoc* **14**, 170–178.

66. Stevens PJ, Syddall HE, Patel HP *et al.* (2012) Is grip strength a good marker of physical performance among community-dwelling older people? *J Nutr Health Aging* **16**, 769–774.

67. Kalyani RR, Stein B, Valiyil R *et al.* (2010) Vitamin D treatment for the prevention of falls in older adults: systematic review and meta-analysis. *J Am Geriatr Soc* **58**, 1299–1310.

68. Toffanello ED, Perissinotto E, Sergi G *et al.* (2012) Vitamin D and physical performance in elderly subjects: the Pro.V.A study. *PLoS ONE* **7**, e34950.

69. Schottker B, Saum KU, Perna L *et al.* (2014) Is vitamin D deficiency a cause of increased morbidity and mortality at older age or simply an indicator of poor health? *Eur J Epidemiol* **29**, 199–210.

70. Uusi-Rasi K, Karkkainen MU & Lamberg-Allardt CJ (2013) Calcium intake in health maintenance - a systematic review. *Food Nutr Res* **57**, doi: 10.3402/fnr.v57i0.21082. Print 2013.

71. Meyer HE, Tverdal A, Falch JA *et al.* (2000) Factors associated with mortality after hip fracture. *Osteoporos Int* **11**, 228–232.

72. Reinhardt T, Horst R, Orf J & Hollis B (1984) A microassay for 1,25-dihydroxyvitamin D not requiring high performance liquid chromatography: application to clinical studies. *J Clin Endocrinol Metab.* **55**, 91–8.

73. Lensmeyer GL, Wiebe DA, Binkely N & Drezner MK (2006) HPLC method for 25-hydroxyvitamin D measurment: comparison with contemporary assays. *Clin Chem* **52**, 1120–6.

Supporting information

Additional Supporting Information may be found online in the supporting information tab for this article:

Appendix S1. Search terms and search hits in Medline, Embase, Pubmed and Web of Science.

Appendix S2. An overview of the physical performance tests used in the randomised controlled trials included in the systematic review and meta-analysis.

Appendix S3. Summary of CONSORT statements for each study included in the systematic review.

Appendix S4. Sensitivity analysis for hand grip strength (HGS) and timed-up-and-go test (TUG).

SUPPLEMENTAL MATERIAL FOR ONLINE ONLY

Appendix S1: Search terms and search hits in Medline, Embase, Pubmed and Web of Science.

Steps	Search term	Search string	Hits Medline	Hits Embase	Hits PubMed	Hits Cochrane Library	Hits Web of Science
1	Vitamin D	"vitamin d [MeSH Terms]" OR "vitamin d [All fields]"					
2	25-OH-D	"25-OH-D [All fields]"					
3	Calcidiol	"calcidiol [All fields]"					
4	Cholecalciferol	"cholecalciferol [All fields]"					
5	Calciferol	"calciferol [All fields]"					
6	ergocalciferol	«ergocalciferol [All fields]»					
7	hydroxycholecalciferols	"hydroxycholecalciferols [All fields]"					
8	1,25-dihydroxyvitamin d2	"1,25-dihydroxyvitamin d2 [All fields]"					
9	dihydroxycholecalciferol	"dihydroxycholecalciferols [All fields]"					
10	1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9						
11	Muscle strength	"Muscle strength [MeSH Terms]"					

			747(109RCT)	1554(166RCT)	959(115RCT)	224 (193 trials)	1040
12	Hand strength	"Hand strength [All fields]"					
13	Weakness	"Weakness [All fields]"					
14	Strength	"Strength [All fields]"					
15	Gait	"gait {MeSH Terms]"					
16	Mobility	"Mobility [All fields]"					
17	11 or 12 or 13 or 14 or 15 or 16						
188	Aging	«Aging [MeSH Terms]"»					
19	Aged	«Aged {MeSH Terms]"»					
20	Frail elderly	«frail elderly {All fields]"»					
21	Elderly	"Elderly [All fields]"					
22	Older adults	"Older adults [All fields]					
23	17 or 18 or 19 or 20 or 21 or 22						
24	10 and 17 and 23						
8	**Results**						

Appendix S2: An overview of the physical performance tests used in the RCTs included in the systematic review and meta-analysis.

Physical performance test	Short description
Hand grip strength (HGS)	Hand grip strength was measured with a hand-held dynamometer [43, 45, 46]. The average of two measurements was used for the analysis (recorded to the nearest half kilogram of force) [45].
Timed up and go (TUG)	Subjects were observed and timed from the instant they rose from an armchair (seat height 48 cm, arm height 68 cm), walked 3 or 4 meters, navigated an obstacle on the floor, and returned to a fully seated position in the chair. Each subject performed the test three times and the fastest of the three trials was finally recorded [41, **1**].
Quadriceps strength	Quadriceps strength was assessed using a strain gauge system attached to a specially constructed chair on which the participants were seated with their hips and knees flexed to approximately 90°. In this position, three maximal voluntary contractions (MVC) were recorded and analysed off-line. The strongest MVC of the dominant leg was used in the data analysis [43, **2**].
Knee extension	Subjects were instructed to complete 5 knee extension repetitions, each separated by 30 seconds, as quickly as possible through their full range of motion at both 40% and 70% of one repetition maximum [28].
Leg muscle strength	Isokinetic muscle strength of the knee flexor and extensor muscles was measured using an isokinetic dynamometer [40].
Gait speed	Subjects completed a 4-meter walk test along a corridor at their normal walking speed. Gait speed was

	calculated by dividing the 4 meters by the time in seconds taken to complete the test [37].
Stair climbing power	The subject was asked to climb a flight of stairs (10 steps, 7.8 cm rise/step) as quickly as possible. It should be done without the use of handrails or any other aid [46]. The stair climbing power was calculated was calculated according to the method by Lazowski et al. [**3**]
The four square step test (FSST)	A square is formed by using 4 canes resting flat on the floor. The subject stands in square number 1 facing square number 2. The aim is to step as fast as possible into each square in the following sequence; square number 2, 3, 4, 1, 4, 3, 2, and 1.The time spent on finishing the sequence is recorded and a score is given according to time spent [**4**].
Leg extension power (LEP)	LEP was measured using a Notting Power Rig. The subject, in seated position with folded arm and a 90-degree knee angle at the start pushed a large foot pedal as hard and fast as possible, setting a flywheel in motion. The best of seven measurements was recorded on both sides [45, **5**].
Modified coop test (COOP)	The COOP is a test in which the maximum walking distance (in meters) achieved in 2 minutes is recorded [45, **6**].
Lower limb muscle strength	Ankle dorsiflexion, knee flexor, knee extensor, hip abductor, hip flexor, hip extensor, and hip adductor strengths were assessed using a strain gauge. The subjects were requested to exert maximum muscle contraction against the strain gauge. The best of three attempts was recorded for each muscle group [44].
Five-times-sit-to-stand-test (FTFFT) or	Using a chair of standard height (40-45 cm), the subjects stood up and sat down five times as rapidly as

Repeated sit-to-stand-test	possible, with the arms folded across the chest. Timing with a digital stopwatch began with the command "go" and ceased when the subject stood up the last time. Two trials were allowed, and the subjects were allowed sufficient time between trials for complete recovery [47, **7**]. The repeated sit-to-stand-test is in principal the same [39].
Short Physical Performance Battery (SPPB)	SPPB includes an assessment of standing balance, a gait speed test (i.e. a timed 4-m walk), and timed rising from a chair and sitting without the use of arms, with 5 repetitions. The SPPB was evaluated using a point scale running from 0 to 12 for combined measures of balance, gait speed, and the ability to rise from a sitting position in a chair, or separately as a continuous variable for the gait speed test [36, **8**].
Functional performance (APFT)	Assessment of lower limb functional performance by determining the time taken to perform four common activities of daily living (50 ft walk, rising from a 42 cm high chair and walking 50 ft, going up and down 13 steps). The sum of the times (in seconds) taken to perform these activities was calculated to determine the Aggregate Functional Performance Time (AFPT) [43, **9**].
Knee flexion strength	Knee flexor strength was measured in kiloponds, with a validated, hand-held isometric method (model DPPH; Chatillon Inc., Greensboro, NC, USA). A nonelastic band was connected to a pull gauge with a continues scale in kiloponds (1 kilopond=10 Newton) [**10**].

References only used in the appendix (in bold writing):

1. Podsiadlo, D. and S. Richardson, *The timed "Up & Go": a test of basic functional mobility for frail elderly persons.* J Am Geriatr Soc, 1991. **39**(2): p. 142-8.

2. Edwards, R.H., et al., *Human skeletal muscle function: description of tests and normal values.* Clin Sci Mol Med, 1977. **52**(3): p. 283-90.

3. Lazowski DA, Ecclestone NA, Myers AM, Paterson DH, Tudor-Locke C, Fitzgerald C, et al. A randomized outcome evaluation of group exercise programs in long-term care institutions. The journals of gerontology Series A, Biological sciences and medical sciences. 1999;54(12):M621-8.

4. Dite W, Temple VA. A clinical test of stepping and change of direction to identify multiple falling older adults. Archives of physical medicine and rehabilitation. 2002;83(11):1566-71.

5. Bassey, E.J. and A.H. Short, *A new method for measuring power output in a single leg extension: feasibility, reliability and validity.* Eur J Appl Physiol Occup Physiol, 1990. **60**(5): p. 385-90.

6. Butland, R.J., et al., *Two-, six-, and 12-minute walking tests in respiratory disease.* Br Med J (Clin Res Ed), 1982. **284**(6329): p. 1607-8.

7. Whitney, S.L., et al., *Clinical measurement of sit-to-stand performance in people with balance disorders: validity of data for the Five-Times-Sit-to-Stand Test.* Phys Ther, 2005. **85**(10): p. 1034-45.

8. Guralnik, J.M., et al., *A short physical performance battery assessing lower extremity function: association with self-reported disability and prediction of mortality and nursing home admission.* J Gerontol, 1994. **49**(2): p. M85-94.

9. Hurley, M.V., J. Rees, and D.J. Newham, *Quadriceps function, proprioceptive acuity and functional performance in healthy young, middle-aged and elderly subjects.* Age Ageing, 1998. **27**(1): p. 55-62.

10. Bischoff HA, Stähelin HB, Dick W, Akos R, Knecht M, Salis C, et al. Effects of vitamin D and calcium supplementation on falls: a randomized controlled trial. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research [Internet]. 2003; 18(2):[343-51 pp.]

Appendix S3: Summary of CONSORT statements for each study included in the systematic review

Section/topic	Item no	Checklist item	Bischof f-Ferrari, 2012	Ceglia, 2013	Dhesi, 2004	Glendenn ing, 2012	Grady, 1991	Janssen, 2009	Kenny, 2003	Lagari, 2010	Lips, 2010	Pfeifer, 2009	Pirotta, 2015	Songpa tansilp, 2009	Wood, 2014	Xia, 2009	Zhu, 2010
Title and abstract																	
	1a	Identification as a randomised trial in the title	-	+	-	+	+	+	-	-	-	-	+	+	+	-	+
	1b	Structured summary of trial design, methods, results and conclusions	+	+/-	+	+	+	+	+	+	+	+	+	+	+	+	+
Introduction																	
Background and objectives	2a	Scientific background and explanation of rationale	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	2b	Specific objectives or hypotheses	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+
Methods																	
Trial design	3a	Description of trial design including allocation ratio	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+

Category	Item	Description												
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	-	-	-	-	-	-	-	-	-	-	-	-
Participants	4a	Eligibility criteria for participants	+	+	+	+	+	+	+	+	+	+	+	+
	4b	Settings and locations where the data were collected	+	+	+	+	+	+	+	+	-	+	+	+
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	+	+	+	+	+	+	+	+	-	+	+	+
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	+	+	+	+	+	+	+	+	+	+	+	+
	6b	Any changes to trial	-	-	-	-	-	-	-	-	-	-	-	-

Section	No.	Description													
		outcomes after the trial commenced, with reasons	+	+	+	-	+	-	+	-	-	+	+	-	+
Sample size	7a	How sample size was determined	-	+	+	-	+	-	+	-	-	+	+	-	+
	7b	When applicable, explanation of any interim analyses and stopping guidelines	-	+	+	-	-	+	-	-	-	-	-	-	-
Randomization:															
Sequence	8a	Method used to generate the random allocation sequence	-	-	+	+	-	+	+	-	-	+	-	+	+
Generation	8b	Type of randomisation; details of any restriction (such as blocking and block size)	-	-	+	+	-	+	+	-	-	+	-	+	+
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the	-	-	+	-	+	+	+	-	+	+	+	+	+

Section	No	Item														
		sequence until interventions were assigned	+	+	+	+	+	-	-	-	+	+	-	+	-	-
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	-	-	+	+	+	-	-	-	+	+	-	+	-	-
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	+	-	+	+	+	-	-	-	+	+	-	+	-	-
	11b	If relevant, description of the similarity of interventions	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	12b	Methods for additional	+	-	+	+	+	+	+	-	+	-	+	+	-	+

		analyses, such as subgroup analyses and adjusted analyses	+	+	+	-	+	+	+	+	+	+	+	+	+
Results															
Participant flow	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	+	+	+	+	+	+	+	+	+	+	+	+	+
	13b	For each group, losses and exclusions after randomisation, together with reasons	-	+	+	+	+	+	+	+	+	+	+	+	+
Recruitment	14a	Dates defining the periods of recruitment and follow-up	-	+	+	-	-	+	-	-	-	-	+	+	-
	14b	Why the trial ended or was stopped	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Baseline data	15	A table showing baseline demographic and clinical characteristics for each	+	+	+	+	+	+	+	+	+	+	+	+	+

Section	No.	Checklist item															
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	-	+	-	+	+	+	+	+	+	+	-	-	+	+	+
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted	-	-	-	+	+	+	-	+	+	+	-	+	-	+	+

Section	No.	Checklist item												
		analyses, distinguishing pre-specified from exploratory			+		+		+	+	+	+	+	+	+
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	-	+	+	-	+	-	-	+	-	+	+	+	+
Discussion														
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	+	+	+	+	+	+	+	+	+	+	+	+	+
Generalizability	21	Generalisability (external validity, applicability) of the trial findings	+	+	+	+	+	+	+	+	+	+	+	+	+
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	-	+	+	+	+	+	+	+	+	+	+	+	+

Other information																
Registration	23	Registration number and name of trial registry	+	-	-	+	-	-	-	-	-	+	-	+	-	+
Protocol	24	Where the full trial protocol can be accessed, if available	-	+	-	-	-	+	+	-	-	+	-	+	+	+
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Appendix S4: Sensitivity analysis for hand grip strength (HGS) and timed up and go test (TUG)

Table 1: Sensitivity analysis for hand grip strength (HGS, kg). The studies mention in the parenthesis is excluded from the analysis.

	No.	Heterogeneity				Test for overall effect		Total (95% CI)					
		Chi²	df	P	I² (%)	Z	p	Intervention	Placebo	Weight	Mean Difference	95% CI	
All studies	7	272,19	8	0,00001	97	0,95	0,34	767	639	100	0,24	-0,25	0,72
Including only studies with normal baseline values of 25OHD (-Xia, Wood and Janssen)	4	3,93	4	0,42	0	25,56	0,00001	487	443	100	0,4	0,37	0,43
Including only studies with a placebo group (-Xia and Lagari)	5	251,51	5	0,00001	98	0,7	0,49	642	538	100	0,22	-0,39	0,83
Including only studies using vitamin D3 (-Xia and Grady)	4	252,2	4	0,00001	98	0,23	0,82	473	429	100	0,08	-0,64	0,81
Including only studies using vitamin D3 and placebo (-Xia, Grady and Lagari)	4	250,04	4	0,00001	98	0,3	0,76	592	490	100	0,1	-0,56	0,77
Including only studies giving Ca-supplements (-Grady, Wood and Lagari)	4	268,04	3	0,00001	99	0,68	0,68	488	468	100	0,22	-0,41	0,85
Including only studies using daily doses of vitamin D (-Glendenning)	6	141,71	7	0,00001	95	0,5	0,62	414	306	100	0,18	-0,53	0,9

Table 2: Sensitivity analysis for timed up and go test (TUG, s). The studies mention in the parenthesis is excluded from the analysis.

	No.	Heterogeneity				Test for overall effect		Total (95% CI)					
		Chi²	df	P	I² (%)	Z	p	Intervention	Placebo	Weight	Mean Difference	95% CI	
All studies	5	82,05	4	0,00001	95	2,93	0,003	638	622	100	0,31	0,1	0,51
Including only studies with normal baseline values of 25OHD (-Zhu and Pirotta)	3	39,7	2	0,00001	95	1,2	0,23	496	478	100	0,51	-0,32	1,34
Including only studies using vitamin D3 (-Zhu)	4	57,89	3	0,00001	95	1,7	0,09	509	490	100	0,16	-0,03	0,35
Including only studies using Ca-suppl. (- Pirotta)	4	64,11	3	0,00001	95	1,68	0,09	625	610	100	0,65	-0,11	1,41
Including only studies using daily doses of vitamin D (-Glendenning)	4	49,57	3	0,00001	94	1,97	0,05	285	289	100	0,68	0	1,36

III

nutrients

MDPI

Article

Limited Benefit of Fish Consumption on Risk of Hip Fracture among Men in the Community-Based Hordaland Health Study

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Abstract: Hip fractures have a high prevalence worldwide. Few studies have investigated whether fish consumption is associated with risk of hip fractures. The objective of the present study was to investigate the effect of fish intake on the subsequent risk of a hip fracture because of the low number of studies on this topic. A community-based prospective cohort study of 2865 men and women from Hordaland county in Norway, born between 1925–1927 and enrolled in the study in 1997–1999. Information on hip fracture cases was extracted from hospital records until 31 December 2009. Baseline information on the intake of fish was obtained from a semi-quantitative food frequency questionnaire. Cox proportional hazard regression models with death as a competing risk were used to evaluate the association of fish intake with risk of hip fracture. During a mean (SD) follow-up time of 9.6 (2.7) years, 226 hip fractures (72 in men, 154 in women) were observed. The mean (SD) fish intake was 48 (25) g/1000 kcal. The association between fish intake and risk of hip fracture was not linear and displayed a threshold, with low intake of fish being associated with an increased risk of hip fracture in men (HR (Hazard Ratio) = 1.84, 95% CI 1.10, 3.08). In this community-based prospective study of men and women, a low intake of fish was associated with the risk of a hip fracture in men.

Keywords: diet; food frequency questionnaire; fish intake; hip fractures

1. Introduction

Hip fracture is a major public health challenge in aging populations worldwide with especially high prevalence in Caucasian populations [1]. Hip fractures substantially increase the risk of morbidity and death in older persons [2,3]. The one-year mortality is about 10–30 percent depending on sex, mental health, and comorbidity burden before the fracture [4–7]. Low bone mineral density (BMD) is the strongest single risk factor for hip fracture and it has been estimated that the risk ratio for hip fracture in men and women increases threefold for each standard deviation (SD) reduction in BMD [8,9]. However, as there are no good biomarkers or clinical signs of changes in BMD, a hip

fracture is often the first sign of low BMD [9]. Postmenopausal women have a more drastic decrease in BMD than men of the same age do. Because women also have a longer life expectancy than men, the majority of low-energy hip fractures occur in women [9].

In addition to low BMD and sex, additional modifiable risk factors such as smoking [10], high alcohol consumption [11], low body weight [12,13], and low physical activity [14] are associated with an increased risk of hip fracture. Diet is discussed as another modifiable risk factor. Much focus in the past has been on single nutrients such as protein [15,16], calcium [17], vitamin D [18], vitamin K [19], and omega-3 polyunsaturated fatty acids (*n*-3 PUFAs) [20]. There is also some evidence that dairy products, as good sources of calcium [21], and a high intake of fruit and vegetables are associated with a lower risk of hip fracture [22,23]. Other food groups, including fish, have been investigated to a lesser degree. Because fish contains several potentially beneficial nutrients, including high quality protein, vitamin D, selenium, iodine, and *n*-3 PUFAs, it is an interesting food group with respect to prevention of hip fractures. Published articles provide mixed results on the association between fish intake and risk of hip fracture; two prospective studies reported a non-significant association [24,25], whereas a case-control study reported a protective effect of high fish intake [26]. In addition, studies have investigated fish as a part of a dietary pattern and reported beneficial but non-significant associations [22] or a beneficial significant effect [27,28]. Thus, the role on fish intake on hip fracture risk is uncertain.

Fish intake shows a high variation among populations, with Spain, Iceland, and Norway on top in the Western world. Total fish intake in these countries is about 50–70 g per day on average and is thus about 3–4 times that of central European countries [29] and the US [24,25].

Studying a Norwegian population of old men and women with among the highest hip fracture prevalence in the world, and with habitually high fish consumption, gives the unique opportunity to investigate total fish intake as well as sub-groups of fish. Thus, the main objective of this study was to investigate the relationship between total, lean, and fatty fish intake and the risk of hip fracture in the Hordaland Health Study (HUSK).

2. Materials and Methods

2.1. Subjects

We used data from the large community-based Hordaland Health Study (HUSK), Western Norway [30]. Participants were born between 1925–1927 and the baseline examinations were conducted during 1997–1999 [31]. Information on hip fractures was collected from the hospitals located in Hordaland County.

2.2. Dietary Assessment

The dietary assessment, questions related to dietary intake of fish and categorization of alcohol consumption, has previously been described in detail [32,33]. Briefly, habitual dietary intake was assessed using a 169-item food frequency questionnaire (FFQ) developed at the Department of Nutrition, Institute of Basic Medical Sciences, University of Oslo. The FFQ has been validated against weighted dietary records and against fatty acid composition in serum phospholipids [34–36]. Participants with an energy intake lower than 700 kcal or 800 kcal and higher than 3600 kcal or 4200 kcal for women and men, respectively, were removed from the analyses, leaving 2865 participants.

In addition to total fish intake (lean, fatty, and processed fish), fish intake was divided into fatty fish (herring, mackerel, salmon, trout, and fish used as spread) and lean fish (cod, pollock, and haddock). The multivariate nutrient density method was used for energy adjustments [37] of all dietary variables and presented as g/1000 kcal (foods and micronutrients) or energy percent (macronutrients). Total marine n-3 PUFA intake was calculated by adding eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA).

One alcohol unit was defined in accordance with the Nordic Nutrition Recommendations 2012 [38]. Sex specific categories of alcohol intake was defined as: 0 = 0 g/day; 1 = women: >0–10 g/day; men: >0–20 g/day; 2 = women: >10–20 g/day; men: >20–30 g/day; 3 = women: >20 g/day; men: >30 g/day.

2.3. Hip Fractures

Collection of information on hip fractures and death has been previously described [39,40], along with a validation of the collection method [41]. Briefly, information on hip fractures was obtained from computerized records containing discharge diagnoses for hip fractures from all hospitalizations between the baseline examinations in HUSK through until 31 December 2009 at the six hospitals in Hordaland County, Norway. Participants were followed until they experienced their first hip fracture or died. A hip fracture was defined as the first fracture of the proximal femur during the observation period. Information on time of death was obtained from the Norwegian Population Register.

2.4. Covariate Assessment

Self-administered question provided information regarding current estrogen therapy, physical activity, and smoking. Physical activity was categorized as by Vinknes et al. [42]. The sum of the scores from categorization of light and hard physical activity was calculated. Categories for light physical activity were 0 (none), 0.25 (<1 h/week), 0.5 (1–2 h/week), or 1.0 ($\geq$3 h/week) and for hard physical activity were 0 (none), 0.5 (<1 h/week), 1.0 (1–2 h/week), or 2.0 ($\geq$3 h/week). Smoking habits were categorized as current smoker, former smoker, and never smoked. Measurements of plasma cotinine were used as a marker of recent nicotine exposure, and participants with cotinine levels $\geq$85 nmol/L were defined as smokers [43,44]. The psychical activity score and plasma cotinine were used in the multivariate models.

2.5. Ethics

HUSK was performed according to the declaration of Helsinki. All participants provided written informed consent. The Regional Committee for Medical and Health Research Ethics approved the study protocol (REC number: 2009/825).

2.6. Statistical Analyses

Continuous variables are presented as means and standard deviation and categorical variables as percentages. Differences in baseline characteristics and dietary intake across sex-specific quartiles were calculated by linear regression for continuous variables and logistic regression for dichotomous variables.

Fish intake (the exposure variable) was introduced in the analyses (a) in four categories (based on its sex-specific quartiles) and (b) as a dichotomous variable (comparing the lower quartile, Q1, to the upper three quartiles Q2 to Q4).

To visually explore the association between fish intake and risk of hip fracture, we used Cox proportional hazards regression model fitting fish intake using restricted cubic splines with three knots. Cox proportional hazards regression models with death as competing risk were used to estimate the risk of hip fractures across fish intake quartiles. The results are presented as hazard ratios (HRs) and 95% confidence interval (CI). Models were adjusted for sex, BMI, plasma concentration of cotinine $\geq$85 nmol/L, physical activity, and energy intake. Further adjustments for intake of vegetable, fruit, dairy, or meat, with vitamin D and calcium intake, or estrogen therapy (in women) were made, none of which changed the strength of the association. In addition to overall analyses, we also conducted sex-specific analyses. Statistical software SPSS for Windows version 25 (IBM, New York, NY, USA) and Stata Statistical Software (Release 15.0, College Station, TX, USA: Stata Corp LLC) were used and a two-sided *p*-value <0.05 was considered statistically significant.

3. Results

Out of 3327 eligible participants, information regarding food intake and hip fractures was available for 2865 (86%).

3.1. Baseline Characteristics and Dietary Intake

Mean (SD) follow-up time was 9.6 years (2.7). Total number of hip fractures was 226 (7.9%), 72 (5.5%) in men and 154 (9.9%) in women. During the study period, 448 men and 305 women died. Death as first event (without experiencing a hip fracture) was observed in 412 (31.3%) men and 257 (16.6%) women. Mean (SD) total fish intake as exposure variable of interest was 48 (25) g/1000 kcal in the entire cohort, with higher consumption in men (51 (25) g/1000 kcal) than in women (45 (25) g/1000 kcal). Lean fish was the largest fish category consumed, followed by fatty fish, and processed fish, both in men and women.

Characteristics of the total cohort stratified by sex-specific quartiles of total fish intake at baseline are presented in Table 1. Both men and women had increasing weight and BMI with higher fish intake. In women, the femoral neck BMD increased with increasing fish intake, but this was not evident in men. Self-reported smoking, plasma cotinine levels, and self-reported high alcohol consumption did not differ across quartiles of fish intake.

Dietary intake of the cohort stratified by sex-specific quartiles of total fish intake at baseline is presented in Table 2. The energy adjusted protein intake (E %) was significantly higher in high consumers of fish compared to low consumers, both in men and women. The same was evident for total vitamin D intake, whereas calcium intake was lower in the high consumers of fish. Although few participants used fish oil supplements, there were more users of this supplement in high consumers of fish. A higher proportion of men with high fish consumption used cod liver oil as supplement compared to the low consumers. Of the food groups consumed, there was an increased intake of vegetables across quartiles in both men and women. In women, the high consumers of fish had a higher intake of fruit and berries compared to the low consumers. Compared to participants with low fish consumption, those with high consumption had lower intake of dairy products.

Table 1. Baseline (1997–1999) characteristics of men and women (age 71–74 years) by sex-specific quartiles of total fish intake. The Hordaland Health study.

	Women					*p* for Trend	Men					*p* for Trend
Total Fish, g/1000 kcal (min–max)	**Total** 1–263	**1st Quartile** (*n* = 387) 1–28	**2nd Quartile** (*n* = 387) 28–41	**3rd Quartile** (*n* = 388) 41–57	**4th Quartile** (*n* = 387) 57–263		**Total** 3–186	**1st Quartile** (*n* = 329) 3–33	**2nd Quartile** (*n* = 329) 33–48	**3rd Quartile** (*n* = 329) 48–65	**4th Quartile** (*n* = 329) 65–186	
Hip fracture incidence, (%)	9.9	11.6	10.1	9.8	8.3	0.128	5.5	7.9	3.6	5.2	5.2	0.234
Femoral neck BMD, g/cm^2	0.763 (0.113)	0.746 (0.108)	0.769 (0.121)	0.766 (0.116)	0.772 (0.106)	0.016	0.901 (0.140)	0.895 (0.127)	0.892 (0.133)	0.913 (0.146)	0.907 (0.152)	0.157
BMI, kg/m^2	26.2 (4.4)	25.8 (4.3)	26.1 (4.4)	26.6 (4.3)	26.3 (4.5)	0.028	26.0 (3.2)	25.8 (3.3)	25.7 (3.0)	25.9 (3.1)	26.5 (3.3)	0.008
Weight, kg	67.7 (11.8)	66.5 (11.6)	67.8 (11.5)	68.5 (11.6)	68.1 (12.4)	0.036	79.5 (11.1)	78.8 (11.2)	78.7 (10.7)	79.1 (10.7)	81.5 (11.6)	0.002
Hard physical activity, %												
≤1 h/week	79.7	81.6	78.1	77.0	82.3	0.900	63.9	67.4	62.8	62.9	62.3	0.209
>1 h/week	20.3	18.4	21.9	23.0	17.7	0.900	36.1	32.6	37.2	37.1	37.7	0.209
Smoking habits, %												
Current smoker	15.2	19.9	11.4	12.6	16.8	0.322	19.5	21.0	20.1	18.2	18.5	0.351
Former smoker	25.0	23.8	22.3	24.7	29.2	0.057	60.1	62.3	56.8	62.3	59.0	0.706
Never smoked	61.2	57.6	67.9	62.6	56.8	0.496	24.0	20.4	25.8	24.0	25.8	0.166
Cotinine ≥85 nmol/L, %	15.3	20.1	11.5	12.7	17.0	0.316	19.4	20.8	20.2	17.7	18.9	0.400
Alcohol categories, % [1]												
None	55.5	64.1	55.3	53.1	49.4	<0.001	32.6	35.6	31.9	30.1	32.8	0.386
Low	39.8	32.6	39.0	43.0	44.7	<0.001	59.8	58.7	60.8	61.4	58.4	0.980
Moderate	4.1	2.8	5.2	3.6	4.9	0.305	3.7	2.4	3.6	3.6	5.2	0.079
High	0.6	0.5	0.5	0.3	1.0	0.455	3.9	3.3	3.6	4.9	3.6	0.655
Estrogen therapy (for women), %	14.8	13.4	14.0	16.8	15.2	0.309	NA	NA	NA	NA	NA	NA

Values represent means (SD). *p* for trend across quartiles of total fish intake; linear regression for continuous variables and logistic regression for dichotomous variables. [1] None: 0 g/day, Low: women: >0–10 g, men: >0–20 g, Moderate: women: >10–20 g, men: >20–30 g, High: women: >20–30 g, men: >30 g. BMD bone mineral density, BMI body mass index, NA not applicable.

Table 2. Baseline (1997–1999) dietary intake of men and women (age 71–74 years) by sex-specific quartiles of total fish intake. The Hordaland Health study.

	Women					p for Trend	Men					p for Trend
	Total	1st Quartile (n = 387)	2nd Quartile (n = 387)	3rd Quartile (n = 388)	4th Quartile (n = 387)		Total	1st Quartile (n = 329)	2nd Quartile (n = 329)	3rd Quartile (n = 329)	4th Quartile (n = 329)	
Total fish, g/1000 kcal (min–max)	1–263	1–28	28–41	41–57	57–263		3–186	3–33	33–48	48–65	65–186	
Total energy, kcal	1605 (491)	1557 (495)	1602 (475)	1646 (492)	1616 (499)	0.050	2059 (594)	2035 (599)	2135 (603)	2078 (553)	1988 (611)	0.180
Protein, E%	15.6 (2.9)	14.8 (2.1)	15.9 (2.0)	16.5 (2.1)	18.4 (2.4)	<0.001	15.5 (1.9)	14.5 (2.0)	15.6 (1.8)	16.3 (1.9)	18.0 (2.2)	<0.001
n-3 long chained PUFA, E% [1]	0.1 (0.1)	0.1 (0.1)	0.1 (0.1)	0.1 (0.1)	0.2 (0.1)	<0.001	0.1 (0.1)	0.1 (0.1)	0.1 (0.1)	0.1 (0.1)	0.2 (0.1)	<0.001
Vitamin D, g/1000 kcal [1]	5.1 (4.3)	4.1 (4.2)	4.7 (4.0)	5.1 (4.1)	6.5 (4.4)	<0.001	5.6 (4.1)	3.9 (3.1)	5.3 (3.9)	6.1 (4.0)	7.2 (4.7)	<0.001
Calcium, g/1000 kcal [1]	450 (138)	467 (151)	454 (132)	447 (134)	434 (131)	0.001	377 (124)	397 (144)	383 (124)	360 (107)	358 (116)	<0.001
Supplements, %												
Fish oil	7.0	3.9	6.7	5.9	11.4	<0.001	5.6	3.6	4.9	6.7	7.3	0.026
Cod liver oil	32.8	31.8	30.5	34.4	34.6	0.245	38.8	28.3	42.2	46.8	38.0	0.005
Vitamin D	2.6	2.8	2.3	2.6	2.6	0.885	1.8	0.9	3.0	0.9	2.4	0.462
Calcium	14.5	10.3	16.3	16.2	15.2	0.067	2.3	2.1	1.8	2.4	2.7	0.509
Food intake, g/1000 kcal												
Total fish	45 (25)	18 (6)	34 (4)	48 (5)	78 (22)	<0.001	51 (25)	23 (7)	41 (4)	55 (5)	85 (20)	<0.001
Lean fish	20 (17)	7 (5)	14 (7)	21 (9)	38 (22)	<0.001	23 (17)	9 (7)	17 (8)	25 (11)	41 (20)	<0.001
Fatty fish	13 (13)	5 (5)	9 (7)	13 (9)	23 (18)	<0.001	15 (13)	6 (6)	12 (8)	16 (10)	26 (17)	<0.001
Processed fish	12 (8)	6 (4)	11 (7)	14 (8)	17 (10)	<0.001	13 (9)	8 (6)	12 (6)	15 (8)	19 (11)	<0.001
Vegetables	115 (73)	94 (72)	114 (70)	117 (66)	135 (79)	<0.001	90 (60)	78 (59)	87 (57)	90 (52)	106 (69)	<0.001
Fruit and berries	151 (101)	138 (104)	158 (104)	152 (90)	157 (104)	0.032	114 (74)	109 (79)	118 (77)	116 (66)	113 (74)	0.542
Meat	40 (20)	34 (18)	41 (20)	42 (20)	42 (20)	<0.001	45 (21)	41 (21)	47 (20)	48 (20)	46 (21)	0.002
Dairy	193 (119)	217 (132)	201 (122)	187 (112)	166 (103)	<0.001	161 (106)	173 (118)	170 (106)	158 (97)	145 (102)	<0.001
Egg	10 (8)	9 (8)	10 (8)	9 (7)	10 (8)	0.520	9 (7)	8 (8)	10 (7)	9 (6)	9 (6)	0.742

Values represent means (SD). p for trend across quartiles of total fish intake; linear regression for continuous variables and logistic regression for dichotomous variables. [1] Intake of calcium and vitamin D includes use of supplements.

3.2. Fish Consumption and the Risk of Hip Fractures

The association of fish intake with hip fracture risk was first explored using fish intake as sex-specific quartiles with separate estimates for total, lean, and fatty fish. The analysis of total fish intake as sex-specific quartiles did not reveal any association between fish intake and the risk of hip fractures, neither in men nor in women (Table 3), nor in the total cohort (data not shown). This result did not change substantially after multiple adjustments. A competing risk analysis of mortality and hip fractures was performed; the results did not change materially (data not shown). A sensitivity analysis was also performed leaving out the non-consumers of lean and fatty fish, but this did not change the results. Separate analyses for lean and fatty fish showed no associations either (data not shown).

The analysis was then extended to include a possible non-linear association of fish intake and hip fracture risk. Low fish intake was associated with higher hip fracture risk although not significantly (Figure 1). A higher risk of hip fractures was also observed when the lowest quartile of fish intake (Q1) was compared to higher fish intake (Q2–Q4) (Table 4). This association was, however, only significant in men. The results remained unchanged after multiple adjustments and competing risk analysis (data not shown).
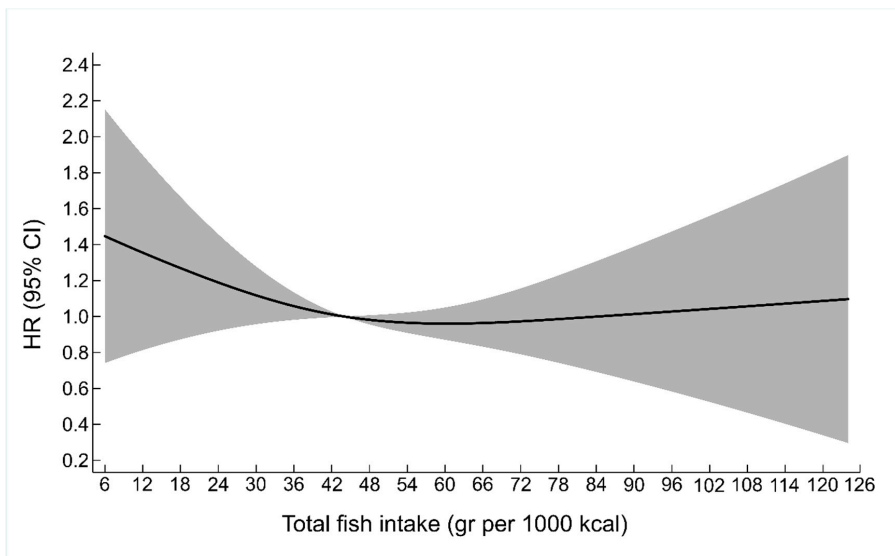
Figure 1. A restricted cubic spline exploring the non-linear association between total fish intake and the risk of hip fracture in the total cohort of men and women (70–74 years old at baseline) from the Hordaland Health Study. HR denotes Hazard Ratio, CI confidence interval.

Table 3. Cox proportional hazards regression analysis of risk of hip fracture according to sex-specific quartiles (Q1–Q4) of baseline total fish intake among men (n = 1315) and women (n = 1548) in the Hordaland Health Study followed from inclusion in 1997–1999 (age 71–74 years) until 31 December 2009.

		Total Fish Intake g/1000 kcal	n Hip Fracture/n Total	Model 1			Model 2			Model 3		
				HR	95% CI	p for Trend	HR	95% CI	p for Trend	HR	95% CI	p for Trend
Men	Q1	23 (3–33)	26/329	1.56	(0.84, 2.87)	0.093	1.51	(0.82, 2.79)	0.091	1.75	(0.88, 3.47)	0.124
	Q2	41 (33–48)	12/329	0.68	(0.33, 1.43)		0.66	(0.31, 1.38)		0.79	(0.35, 1.78)	
	Q3	55 (48–65)	17/329	0.94	(0.48, 1.83)		0.91	(0.46, 1.79)		1.06	(0.50, 2.24)	
	Q4	85 (65–186)	17/329	1.00 (ref)			1.00 (ref)			1.00 (ref)		
Women	Q1	18 (1–28)	45/387	1.39	(0.87, 2.19)	0.554	1.34	(0.85, 2.21)	0.649	1.47	(0.88, 2.44)	0.499
	Q2	34 (28–41)	39/387	1.23	(0.77, 1.96)		1.22	(0.76, 1.94)		1.37	(0.82, 2.30)	
	Q3	48 (41–57)	38/388	1.18	(0.74, 1.89)		1.20	(0.75, 1.92)		1.32	(0.76, 2.21)	
	Q4	78 (57–263)	32/387	1.00 (ref)			1.00 (ref)			1.00 (ref)		

Model 1: unadjusted; Model 2: adjusted for BMI (cont.); Model 3: adjusted for BMI (cont.), energy intake (cont.), nicotine exposure (plasma cotinine ≥ 85 nmol/L, yes/no), and physical activity score (none/low/moderate/high). HR denotes Hazard Ratio.

Table 4. Cox proportional hazards regression analysis of risk of hip fracture according to total fish intake comparing Q1 to Q2–Q4 for the total cohort (n = 2865), men (n = 1315), and women (n = 1548) from the Hordaland Health Study followed from inclusion in 1997–1999 (age 71–74 years) until 31 December 2009.

		Total Fish Intake g/1000 kcal	Model 1			Model 2			Model 3		
			HR	95% CI	p	HR	95% CI	p	HR	95% CI	p
Total cohort	Q1	21 (0.9–33)	1.40	(1.06, 1.85)	0.020	1.39	(1.05, 1.84)	0.022	1.36	(1.00, 1.85)	0.048
	Q2–Q4	57 (28–263)	1.00 (ref)			1.00 (ref)			1.00 (ref)		
Men	Q1	23 (3–33)	1.78	(1.10, 2.88)	0.018	1.77	(1.10, 2.87)	0.019	1.84	(1.10, 3.08)	0.021
	Q2–Q4	60 (33–186)	1.00 (ref)			1.00 (ref)			1.00 (ref)		
Women	Q1	18 (1–28)	1.23	(0.87, 1.74)	0.250	1.18	(0.83, 1.67)	0.350	1.20	(0.82, 1.75)	0.359
	Q2–Q4	53 (28–263)	1.00 (ref)			1.00 (ref)			1.00 (ref)		

Model 1: unadjusted; Model 2: adjusted for sex for the total cohort or BMI (cont.) for the different sex groups; Model 3: adjusted for BMI (cont.), physical activity score (none/low/moderate/high), nicotine exposure (plasma cotinine ≥ 85 nmol/L, yes/no), and energy intake (cont.) (and sex in the total cohort). HR denotes Hazard Ratio.

4. Discussion

Our results suggest the existence of a threshold of fish intake below which the risk of hip fracture appears to increase in older men. This finding is not altered by multiple adjustments and examining death as a possible competing risk for hip fracture.

4.1. Comparison with Other Studies

Other prospective studies that have investigated the association with fish intake and the risk of hip fracture did not find significant associations [24,25,45]. A major difference among studies is the amount of fish consumed. In our study, the fish consumption was high. Participants in the previous studies would be categorized mainly in the lowest quartile of fish intake in our study [24,25,45]. However, comparison between studies is difficult because other scientists have categorized fish intake differently [24–26]. The amount of fish consumed in the present study was high enough to allow analysis of sub-groups of fish intake (lean and fatty fish) separately. However, no association with the risk of hip fracture was observed in the sub-groups. Other studies have performed sub-group analysis of fish intake, but the categorization differs. As an example, some studies included shellfish in the fish category [25,26]. Other differences between the studies include methodological differences in dietary assessment and adjustment for covariates, and assessment of servings. Another methodological issue is evaluation of fish intake as part of a general dietary pattern [22,27,28]. In addition to the studies mentioned above that have been conducted in predominantly Caucasian populations, there are also studies from Asia [26,46,47]. Asian and Caucasian populations differ genetically, but also in lifestyle and dietary intake. However, the main conclusion from the studies in Asian populations was that there is no or only a weak protective association between fish intake and risk of hip fracture.

Assessment of dietary data is challenging, and methodological differences may account for different findings. Fish intake in most of these studies was assessed using a FFQ [24,25,27,28]. However, in the European Prospective Investigation into Cancer (EPIC)-study FFQ, diet history, and 24 h recall [22] were used. In the Japanese cohort [46] a non-specified dietary questionnaire was used. Amounts of fish in these studies were defined either in servings/week [24,25], times/week or month [45], or above and below median intake [22,27], as part of a dietary pattern [47], portions/day [28], or not accounted for [46], which makes any comparison difficult. Moreover, only a few studies reported energy-adjusted fish intake, and those who did used the energy residual method [25,26,28,45,47].

4.2. Fish and Hip Fracture

When evaluating the risk associated with a single food group, one always has to take into account the interactions between food groups. High fish consumers had a higher intake of meat, vegetables, and in women, fruit and berries, and lower intake of dairy. In our study, high fish consumers had a higher intake of meat and vegetables, and among women, high fish consumers ate more fruit and berries and less dairy food. Similar results have been reported from a Spanish study that had a comparable fish intake to our study. Protective effects of high vegetable and fruit consumption have been reported on the risk of hip fracture [23,48]. For other food groups, there is some uncertainty regarding an association. This is especially true for dairy products, which is a good source of calcium, although the association with the risk of hip fracture is uncertain [49]. In the present study, adjustment for dairy intake did not change the results.

Fish is a good source of n3 PUFAs that have been proposed to be protective [20,25,50,51]. In our population of Western Norway, the association of fish and PUFA intake may have been modified by the high frequency of cod liver consumption. In addition to n3 PUFAs, cod liver oil is also a source of vitamin D. In Norway, it is recommended to those older than 65 years as a vitamin D supplement. In national surveys, about half of the population followed this advice [52]. However, many older persons do not consume cod liver oil throughout the year, but information on periods of supplement use is lacking in HUSK.

Similar to other studies [22,26], we observed a stronger association of fish intake with hip fractures in men than in women. The association of low intake with increased risk was only significant in men, which could be due to lower baseline risk in men and a larger variation in fish consumption. These results may suggest there are other factors aside from fish consumption that are important for the prevention of hip fracture. In the same population, we previously reported an association between fish intake and BMD in women [32] whilst investigating fish intake as a continuous variable and in sex-specific quartiles. However, this did not affect the association of fish intake and hip fractures. The same was evident for the Framingham Osteoporosis study reporting an association between fish intake and BMD [53] but not with the risk of hip fracture [25]. In the Cardiovascular Health Study [24], there was no association between fish intake and either BMD or hip fracture risk.

4.3. Strength and Limitations

The strength of our community-based study is its large sample size with a narrow age range (70–74 years), a high habitual fish consumption, and a long observation period. The fish intake reported in our study was similar to intake obtained in the Norwegian dietary survey from 1997 [52] of the 60–79 age group, suggesting that the estimate was valid. The main limitation was that fish intake and data on potential confounders were only assessed at baseline, and not during the follow-up. However, dietary habits seldom change rapidly, especially among older persons living at home [54]. Thus, the intakes reported are likely to represent habits extended throughout a longer period of life.

5. Conclusions

In this large community-based study of men and women with a high habitual fish consumption, we found a significantly increased risk of hip fracture among men in the lowest quartile compared to the upper three quartiles of fish intake.

References

1. Ballane, G.; Cauley, J.A.; Luckey, M.M.; Fuleihan Gel, H. Secular trends in hip fractures worldwide: Opposing trends east versus west. *J. Bone Miner. Res.* **2014**, *29*, 1745–1755. [CrossRef] [PubMed]
2. Wolinsky, F.D.; Fitzgerald, J.F.; Stump, T.E. The effect of hip fracture on mortality, hospitalization, and functional status: A prospective study. *Am. J. Public Health* **1997**, *87*, 398–403. [CrossRef] [PubMed]
3. Bentler, S.E.; Liu, L.; Obrizan, M.; Cook, E.A.; Wright, K.B.; Geweke, J.F.; Chrischilles, E.A.; Pavlik, C.E.; Wallace, R.B.; Ohsfeldt, R.L.; et al. The aftermath of hip fracture: Discharge placement, functional status change, and mortality. *Am. J. Epidemiol.* **2009**, *170*, 1290–1299. [CrossRef] [PubMed]
4. Panula, J.; Pihlajamaki, H.; Mattila, V.M.; Jaatinen, P.; Vahlberg, T.; Aarnio, P.; Kivela, S.L. Mortality and cause of death in hip fracture patients aged 65 or older: A population-based study. *BMC Musculoskelet. Disord.* **2011**, *12*, 105. [CrossRef] [PubMed]
5. LeBlanc, E.S.; Hillier, T.A.; Pedula, K.L.; Rizzo, J.H.; Cawthon, P.M.; Fink, H.A.; Cauley, J.A.; Bauer, D.C.; Black, D.M.; Cummings, S.R.; et al. Hip fracture and increased short-term but not long-term mortality in healthy older women. *Arch. Intern. Med.* **2011**, *171*, 1831–1837. [CrossRef] [PubMed]

6. Pedersen, A.B.; Ehrenstein, V.; Szépligeti, S.; Lunde, A.; Lagerros, Y.T.; Westerlund, A.; Tell, G.S.; Sørensen, H.T. 35-year trends in first-time hospitalization for hip fracture, one year mortality, and the prognostic impact of comorbidity: A danish nationwide cohort study, 1980–2014. *Epidemiology (Camb. MA)* **2017**, *28*, 898–905. [CrossRef] [PubMed]

7. Omsland, T.K.; Emaus, N.; Tell, G.S.; Magnus, J.H.; Ahmed, L.A.; Holvik, K.; Center, J.; Forsmo, S.; Gjesdal, C.G.; Schei, B.; et al. Mortality following the first hip fracture in Norwegian women and men (1999–2008). A norepos study. *Bone* **2014**, *63*, 81–86. [CrossRef] [PubMed]

8. Meyer, H.E.; Tverdal, A.; Falch, J.A. Risk factors for hip fracture in middle-aged Norwegian women and men. *Am. J. Epidemiol* **1993**, *137*, 1203–1211. [CrossRef] [PubMed]

9. Cummings, S.R.; Melton, L.J. Epidemiology and outcomes of osteoporotic fractures. *Lancet* **2002**, *359*, 1761–1767. [CrossRef]

10. Kanis, J.A.; Johnell, O.; Oden, A.; Johansson, H.; De Laet, C.; Eisman, J.A.; Fujiwara, S.; Kroger, H.; McCloskey, E.V.; Mellstrom, D.; et al. Smoking and fracture risk: A meta-analysis. *Osteoporos. Int.* **2005**, *16*, 155–162. [CrossRef] [PubMed]

11. Berg, K.M.; Kunins, H.V.; Jackson, J.L.; Nahvi, S.; Chaudhry, A.; Harris, K.A., Jr.; Malik, R.; Arnsten, J.H. Association between alcohol consumption and both osteoporotic fracture and bone density. *Am. J. Med.* **2008**, *121*, 406–418. [CrossRef] [PubMed]

12. Mpalaris, V.; Anagnostis, P.; Goulis, D.G.; Iakovou, I. Complex association between body weight and fracture risk in postmenopausal women. *Obes. Rev.* **2015**, *16*, 225–233. [CrossRef] [PubMed]

13. Sogaard, A.J.; Holvik, K.; Omsland, T.K.; Tell, G.S.; Dahl, C.; Schei, B.; Meyer, H.E. Age and sex differences in body mass index as a predictor of hip fracture: A norepos study. *Am. J. Epidemiol.* **2016**, *184*, 510–519. [CrossRef] [PubMed]

14. Gillespie Lesley, D.; Robertson, M.C.; Gillespie William, J.; Sherrington, C.; Gates, S.; Clemson Lindy, M.; Lamb Sarah, E. Interventions for preventing falls in older people living in the community. In *Cochrane Database of Systematic Reviews*; John Wiley & Sons, Ltd.: Hoboken, NJ, USA, 2012.

15. Shams-White, M.M.; Chung, M.; Du, M.; Fu, Z.; Insogna, K.L.; Karlsen, M.C.; LeBoff, M.S.; Shapses, S.A.; Sackey, J.; Wallace, T.C.; et al. Dietary protein and bone health: A systematic review and meta-analysis from the national osteoporosis foundation. *Am. J. Clin. Nutr.* **2017**, *105*, 1528–1543. [CrossRef] [PubMed]

16. Sahni, S.; Cupples, L.A.; McLean, R.R.; Tucker, K.L.; Broe, K.E.; Kiel, D.P.; Hannan, M.T. Protective effect of high protein and calcium intake on the risk of hip fracture in the framingham offspring cohort. *J. Bone Miner. Res.* **2010**, *25*, 2770–2776. [CrossRef] [PubMed]

17. Bolland, M.J.; Leung, W.; Tai, V.; Bastin, S.; Gamble, G.D.; Grey, A.; Reid, I.R. Calcium intake and risk of fracture: Systematic review. *BMJ* **2015**, *351*, h4580. [CrossRef] [PubMed]

18. Avenell, A.; Mak Jenson, C.S.; O'Connell, D. Vitamin D and Vitamin D analogues for preventing fractures in post-men. In *Cochrane Database of Systematic Reviews*; John Wiley & Sons, Ltd.: Hoboken, NJ, USA, 2014.

19. Cockayne, S.; Adamson, J.; Lanham-New, S.; Shearer, M.J.; Gilbody, S.; Torgerson, D.J. Vitamin K and the prevention of fractures: Systematic review and meta-analysis of randomized controlled trials. *Arch. Intern. Med.* **2006**, *166*, 1256–1261. [CrossRef] [PubMed]

20. Orchard, T.S.; Cauley, J.A.; Frank, G.C.; Neuhouser, M.L.; Robinson, J.G.; Snetselaar, L.; Tylavsky, F.; Wactawski-Wende, J.; Young, A.M.; Lu, B.; et al. Fatty acid consumption and risk of fracture in the women's health initiative. *Am. J. Clin. Nutr.* **2010**, *92*, 1452–1460. [CrossRef] [PubMed]

21. Weaver, C.M.; Gordon, C.M.; Janz, K.F.; Kalkwarf, H.J.; Lappe, J.M.; Lewis, R.; O'Karma, M.; Wallace, T.C.; Zemel, B.S. The national osteoporosis foundation's position statement on peak bone mass development and lifestyle factors: A systematic review and implementation recommendations. *Osteoporos. Int.* **2016**, *27*, 1281–1286. [CrossRef] [PubMed]

22. Benetou, V.; Orfanos, P.; Pettersson-Kymmer, U.; Bergstrom, U.; Svensson, O.; Johansson, I.; Berrino, F.; Tumino, R.; Borch, K.B.; Lund, E.; et al. Mediterranean diet and incidence of hip fractures in a European cohort. *Osteoporos. Int.* **2013**, *24*, 1587–1598. [CrossRef] [PubMed]

23. Byberg, L.; Bellavia, A.; Orsini, N.; Wolk, A.; Michaelsson, K. Fruit and vegetable intake and risk of hip fracture: A cohort study of Swedish men and women. *J. Bone Miner. Res.* **2015**, *30*, 976–984. [CrossRef] [PubMed]

24. Virtanen, J.K.; Mozaffarian, D.; Cauley, J.A.; Mukamal, K.J.; Robbins, J.; Siscovick, D.S. Fish consumption, bone mineral density, and risk of hip fracture among older adults: The cardiovascular health study. *J. Bone Miner. Res.* **2010**, *25*, 1972–1979. [CrossRef] [PubMed]

25. Farina, E.K.; Kiel, D.P.; Roubenoff, R.; Schaefer, E.J.; Cupples, L.A.; Tucker, K.L. Dietary intakes of arachidonic acid and alpha-linolenic acid are associated with reduced risk of hip fracture in older adults. *J. Nutr.* **2011**, *141*, 1146–1153. [CrossRef] [PubMed]

26. Fan, F.; Xue, W.Q.; Wu, B.H.; He, M.G.; Xie, H.L.; Ouyang, W.F.; Tu, S.L.; Chen, Y.M. Higher fish intake is associated with a lower risk of hip fractures in Chinese men and women: A matched case-control study. *PLoS ONE* **2013**, *8*, e56849. [CrossRef] [PubMed]

27. Haring, B.; Crandall, C.J.; Wu, C.; LeBlanc, E.S.; Shikany, J.M.; Carbone, L.; Orchard, T.; Thomas, F.; Wactawaski-Wende, J.; Li, W.; et al. Dietary patterns and fractures in postmenopausal women: Results from the women's health initiative. *JAMA Intern. Med.* **2016**, *176*, 645–652. [CrossRef] [PubMed]

28. Byberg, L.; Bellavia, A.; Larsson, S.C.; Orsini, N.; Wolk, A.; Michaelsson, K. Mediterranean diet and hip fracture in Swedish men and women. *J. Bone Miner. Res.* **2016**, *31*, 2098–2105. [CrossRef] [PubMed]

29. Welch, A.A.; Lund, E.; Amiano, P.; Dorronsoro, M.; Brustad, M.; Kumle, M.; Rodriguez, M.; Lasheras, C.; Janzon, L.; Jansson, J.; et al. Variability of fish consumption within the 10 European countries participating in the European investigation into cancer and nutrition (epic) study. *Public Health Nutr.* **2002**, *5*, 1273–1285. [CrossRef] [PubMed]

30. Refsum, H.; Nurk, E.; Smith, A.D.; Ueland, P.M.; Gjesdal, C.G.; Bjelland, I.; Tverdal, A.; Tell, G.S.; Nygard, O.; Vollset, S.E. The hordaland homocysteine study: A community-based study of homocysteine, its determinants, and associations with disease. *J. Nutr.* **2006**, *136*, 1731S–1740S. [CrossRef] [PubMed]

31. The Hordaland Health Studies. Available online: https://husk-en.w.uib.no/ (accessed on 5 July 2018).

32. Rosendahl-Riise, H.; Karlsson, T.; Drevon, C.A.; Apalset, E.M.; Nygard, O.K.; Tell, G.S.; Dierkes, J. Total and lean fish intake is positively associated with bone mineral density in older women in the community-based hordaland health study. *Eur. J. Nutr.* **2018**. [CrossRef] [PubMed]

33. Nurk, E.; Drevon, C.A.; Refsum, H.; Solvoll, K.; Vollset, S.E.; Nygard, O.; Nygaard, H.A.; Engedal, K.; Tell, G.S.; Smith, A.D. Cognitive performance among the elderly and dietary fish intake: The hordaland health study. *Am. J. Clin. Nutr.* **2007**, *86*, 1470–1478. [CrossRef] [PubMed]

34. Nes, M.; Frost Andersen, L.; Solvoll, K.; Sandstad, B.; Hustvedt, B.E.; Lovo, A.; Drevon, C.A. Accuracy of a quantitative food frequency questionnaire applied in elderly Norwegian women. *Eur. J. Clin. Nutr.* **1992**, *46*, 809–821. [PubMed]

35. Andersen, L.F.; Solvoll, K.; Johansson, L.R.; Salminen, I.; Aro, A.; Drevon, C.A. Evaluation of a food frequency questionnaire with weighed records, fatty acids, and alpha-tocopherol in adipose tissue and serum. *Am. J. Epidemiol* **1999**, *150*, 75–87. [CrossRef] [PubMed]

36. Andersen, L.F.; Solvoll, K.; Drevon, C.A. Very-long-chain n-3 fatty acids as biomarkers for intake of fish and n-3 fatty acid concentrates. *Am. J. Clin. Nutr.* **1996**, *64*, 305–311. [CrossRef] [PubMed]

37. Willett, W.C.; Howe, G.R.; Kushi, L.H. Adjustment for total energy intake in epidemiologic studies. *Am. J. Clin. Nutr.* **1997**, *65*, 1220S–1228S, discussion 1229S–1231S. [CrossRef] [PubMed]

38. Nordic Council of Ministers. *Nordic Nutritional Recommendations 2012–Integrating Nutrition and Physical Activity*; Nordic Council of Ministers: Copenhagen, Denmark, 2012; pp. 1–267.

39. Gjesdal, C.G.; Vollset, S.E.; Ueland, P.M.; Refsum, H.; Meyer, H.E.; Tell, G.S. Plasma homocysteine, folate, and Vitamin B 12 and the risk of hip fracture: The hordaland homocysteine study. *J. Bone Miner. Res.* **2007**, *22*, 747–756. [CrossRef] [PubMed]

40. Apalset, E.M.; Gjesdal, C.G.; Eide, G.E.; Tell, G.S. Intake of Vitamin K1 and K2 and risk of hip fractures: The hordaland health study. *Bone* **2011**, *49*, 990–995. [CrossRef] [PubMed]

41. Lofthus, C.M.; Osnes, E.K.; Falch, J.A.; Kaastad, T.S.; Kristiansen, I.S.; Nordsletten, L.; Stensvold, I.; Meyer, H.E. Epidemiology of hip fractures in Oslo, Norway. *Bone* **2001**, *29*, 413–418. [CrossRef]

42. Vinknes, K.J.; Elshorbagy, A.K.; Nurk, E.; Drevon, C.A.; Gjesdal, C.G.; Tell, G.S.; Nygard, O.; Vollset, S.E.; Refsum, H. Plasma stearoyl-coa desaturase indices: Association with lifestyle, diet, and body composition. *Obesity* **2013**, *21*, E294–E302. [CrossRef] [PubMed]

43. SRNT Subcommittee on Biochemical Verification. Biochemical verification of tobacco use and cessation. *Nicotine Tob. Res.* **2002**, *4*, 149–159.

44. Oyen, J.; Gram Gjesdal, C.; Nygard, O.K.; Lie, S.A.; Meyer, H.E.; Apalset, E.M.; Ueland, P.M.; Pedersen, E.R.; Midttun, O.; Vollset, S.E.; et al. Smoking and body fat mass in relation to bone mineral density and hip fracture: The hordaland health study. *PLoS ONE* **2014**, *9*, e92882. [CrossRef] [PubMed]

45. Feskanich, D.; Willett, W.C.; Colditz, G.A. Calcium, Vitamin D, milk consumption, and hip fractures: A prospective study among postmenopausal women. *Am. J. Clin. Nutr.* **2003**, *77*, 504–511. [CrossRef] [PubMed]

46. Fujiwara, S.; Kasagi, F.; Yamada, M.; Kodama, K. Risk factors for hip fracture in a japanese cohort. *J. Bone Miner. Res.* **1997**, *12*, 998–1004. [CrossRef] [PubMed]

47. Zeng, F.F.; Wu, B.H.; Fan, F.; Xie, H.L.; Xue, W.Q.; Zhu, H.L.; Chen, Y.M. Dietary patterns and the risk of hip fractures in elderly Chinese: A matched case-control study. *J. Clin. Endocrinol. Metab.* **2013**, *98*, 2347–2355. [CrossRef] [PubMed]

48. Benetou, V.; Orfanos, P.; Feskanich, D.; Michaelsson, K.; Pettersson-Kymmer, U.; Eriksson, S.; Grodstein, F.; Wolk, A.; Bellavia, A.; Ahmed, L.A.; et al. Fruit and vegetable intake and hip fracture incidence in older men and women: The chances project. *J. Bone Miner. Res.* **2016**, *31*, 1743–1752. [CrossRef] [PubMed]

49. Bian, S.; Hu, J.; Zhang, K.; Wang, Y.; Yu, M.; Ma, J. Dairy product consumption and risk of hip fracture: A systematic review and meta-analysis. *BMC Public Health* **2018**, *18*, 165. [CrossRef] [PubMed]

50. Benetou, V.; Orfanos, P.; Zylis, D.; Sieri, S.; Contiero, P.; Tumino, R.; Giurdanella, M.C.; Peeters, P.H.; Linseisen, J.; Nieters, A.; et al. Diet and hip fractures among elderly Europeans in the epic cohort. *Eur. J. Clin. Nutr.* **2011**, *65*, 132–139. [CrossRef] [PubMed]

51. Harris, T.B.; Song, X.; Reinders, I.; Lang, T.F.; Garcia, M.E.; Siggeirsdottir, K.; Sigurdsson, S.; Gudnason, V.; Eiriksdottir, G.; Sigurdsson, G.; et al. Plasma phospholipid fatty acids and fish-oil consumption in relation to osteoporotic fracture risk in older adults: The age, gene/environment susceptibility study. *Am. J. Clin. Nutr.* **2015**, *101*, 947–955. [CrossRef] [PubMed]

52. Johansson, L.; Solvoll, K. *Norkost 1997–Landsomfattende Kostholdsundersøkelse Blant Menn og Kvinner i Alderen 16–79 år.*; Statens Råd for Ernæring og Fysisk Aktivitet.: Oslo, Norway, 1999.

53. Farina, E.K.; Kiel, D.P.; Roubenoff, R.; Schaefer, E.J.; Cupples, L.A.; Tucker, K.L. Protective effects of fish intake and interactive effects of long-chain polyunsaturated fatty acid intakes on hip bone mineral density in older adults: The framingham osteoporosis study. *Am. J. Clin. Nutr.* **2011**, *93*, 1142–1151. [CrossRef] [PubMed]

54. Newby, P.K.; Weismayer, C.; Akesson, A.; Tucker, K.L.; Wolk, A. Long-term stability of food patterns identified by use of factor analysis among Swedish women. *J. Nutr.* **2006**, *136*, 626–633. [CrossRef] [PubMed]

IV

V

HVA SPISER DU?

I dette skjemaet spør vi om dine spisevaner slik de **vanligvis** er.
Vi er klar over at kostholdet varierer fra dag til dag. Prøv derfor
så godt du kan å gi et **"gjennomsnitt"** av dine spisevaner. Ha det
siste året i tankene når du fyller ut skjemaet. Der du er usikker,
anslå svaret.

Skjemaet skal leses av en maskin, og derfor er det viktig at du
setter et tydelig kryss i avmerket rute.

Riktig markering er slik: ⊠

Bruk helst bløt blyant. Feil kan da rettes med viskelær.
Kulepenn og svart tusjpenn kan også brukes.

Av hensyn til den maskinelle lesingen pass på at arkene ikke blir brettet.

Alle svar vil bli behandlet strengt fortrolig.

EKSEMPEL PÅ UTFYLLING AV SPØRSMÅL 1.

Kari Nordmann spiser daglig 5 skiver brød og ett knekkebrød. Hun spiser vanligvis kneippbrød, men i helgene blir det en del loff. I tillegg spiser hun ett knekkebrød hver dag. Hun fyller ut første spørsmål slik:

1. HVOR MYE BRØD PLEIER DU Å SPISE?

Legg sammen det du bruker til alle måltider i løpet av en dag.

(1/2 rundstykke = 1 skive, 1 baguett = 5 skiver, 1 ciabatta = 4 skiver)

Antall skiver pr. dag

	0	1/2	1	2	3	4	5	6	7	8	9	10	11	12+
Fint brød (loff, baguetter, fine rundstykker o.l.)	☐	☐	☒	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Mellomgrovt brød (lys helkorn, lys kneipp, lyst hj.bakt o.l.)	☐	☐	☐	☐	☐	☒	☐	☐	☐	☐	☐	☐	☐	☐
Grovt brød (fiberkneipp, mørk kneipp, mørkt hj.bakt o.l.)	☒	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Knekkebrød (kavring, grov skonrok o.l.)	☐	☐	☒	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

Sum skiver pr. dag = __6__
Antall skiver pr. uke: __6 x 7__ = _42_ Tallet brukes i spørsmål 5.

1. HVOR MYE BRØD PLEIER DU Å SPISE?

Legg sammen det du bruker til alle måltider i løpet av en dag.

(1/2 rundstykke = 1 skive, 1 baguett = 5 skiver, 1 ciabatta = 4 skiver)

	Antall skiver pr. dag													
	0	1/2	1	2	3	4	5	6	7	8	9	10	11	12+
Fint brød (loff, baguetter, fine rundstykker o.l.)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Mellomgrovt brød (lys helkorn, lys kneipp, lyst hj.bakt o.l.)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Grovt brød (fiberkneipp, mørk kneipp, mørkt hj.bakt o.l.)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Knekkebrød (kavring, grov skonrok o.l.)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

Sum skiver pr. dag = _____
Antall skiver pr. uke: _____ x 7 = _____. Tallet brukes i spørsmål 5.

2. HVA PLEIER DU Å SMØRE PÅ BRØDET?

Merk av både for hverdag og helg, selv om du bruker det samme.

Hverdager		Lørdager, søndager
☐	Bruker ikke	☐
☐	Smør (meierismør)	☐
☐	Bremykt, Smøregod	☐
☐	Brelett	☐
☐	Soft, soyamargarin (pakke, beger)	☐
☐	Solsikke	☐
☐	Oliven	☐
☐	Vita	☐
☐	Olivero	☐
☐	Omega	☐
☐	Soft light	☐
☐	Vita lett	☐
☐	Annen margarin	☐

3. OM DU BRUKER FETT PÅ BRØD, HVOR MYE BRUKER DU?

En porsjonspakning på 12 g rekker til antall skiver

1	☐
2	☐
3	☐
4	☐
5	☐

4. MELK SOM DRIKK

(1 glass = 1,5 dl)

	Drikker sjelden/ ikke	Antall glass pr. dag								
		1/2	1	2	3	4	5	6	7	8+
Helmelk, søt, sur	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Lettmelk, søt, sur	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Lettmelk, ekstra lett	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Skummet melk, søt, sur	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

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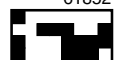
5. PÅLEGGSSORTER

Bruk sum skiver pr. uke fra spørsmål 1.

Til antall skiver pr. uke

	0	1/2	1	2-3	4-5	6-7	8-14	15-21	22-28	29-35	36+
Brun ost, prim	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Hvit ost, helfet, 27% fett (Jarlsberg, Norvegia o.l., smøreost; eske, tube)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Hvit ost, halvfet, 16% fett (Jarlsberg, Norvegia o.l., smøreost; eske, tube)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Ost med mer enn 27% fett (kremoster, Normanna, Ridderost)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

	0	1/2	1	2-3	4-5	6-7	8-14	15-21	22-28	29-35	36+
Leverpostei, vanlig	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Leverpostei, mager	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Servelat, vanlig	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Lett servelat, kalverull, kokt skinke, okserull o.l.	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Salt pølse, spekepølse (fårepølse, salami o.l.)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

	0	1/2	1	2-3	4-5	6-7	8-14	15-21	22-28	29-35	36+
Kaviar	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Makrell i tomat, røkt makrell	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Sardiner, sursild, ansjos o.l.	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Laks, ørret	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Reker, krabbe	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

	0	1/2	1	2-3	4-5	6-7	8-14	15-21	22-28	29-35	36+
Syltetøy, marmelade, frysetøy	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Honning, sirup, sjokolade-, nøttepålegg	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

	0	1/2	1	2-3	4-5	6-7	8-14	15-21	22-28	29-35	36+
Grønnsaker som pålegg (agurk, tomat o.l.)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Frukt som pålegg (banan, eple o.l.)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Salater med majones	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Majones på smørbrød	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

6. EGG

Antall pr. uke

	0	Mindre enn 1	1	2	3-4	5-6	7	8+
(kokt, stekt, eggerøre, omelett)	☐	☐	☐	☐	☐	☐	☐	☐

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7. FROKOSTGRYN, GRØT OG YOGHURT

Svar enten pr. måned **eller** pr. uke. <1 betyr sjeldnere enn 1 gang.

	Gang pr. måned					Gang pr. uke						Mengde pr. gang			
	0	<1	1	2	3	1	2-3	4-5	6-7	8+					
Havregryn, kornblandinger (4-korn, usøtet müsli o.l.)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(dl)	1 ☐	1 1/2 ☐	2 ☐	3+ ☐
Cornflakes, puffet ris, havrenøtter o.l.	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(dl)	1 ☐	1 1/2 ☐	2 ☐	3+ ☐
Havregrøt	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(dl)	1-2 ☐	3-4 ☐	5-6 ☐	7+ ☐
Sukker til frokostgryn, grøt	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(ts)	1 ☐	2 ☐	3-4 ☐	5+ ☐
Yoghurt, naturell, frukt	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(beger)	1/2 ☐	1 ☐	1 1/2 ☐	2+ ☐
Lettyoghurt	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(beger)	1/2 ☐	1 ☐	1 1/2 ☐	2+ ☐
Go´morgen yoghurt inkl. müsli	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(beger)	1/2 ☐	1 ☐	1 1/2 ☐	2+ ☐
Melk søt, sur på gryn, grøt og dessert	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(dl)	3/4 ☐	1 ☐	2 ☐	3+ ☐

8. KAFFE OG TE

(1 kopp kaffe = 1,2 dl   1 kopp te = 2 dl)

	Drikker ikke/ikke daglig	Antall kopper pr. dag							
		1/2	1	2	3-4	5-6	7-8	9-10	11+
Kaffe, kokt	☐	☐	☐	☐	☐	☐	☐	☐	☐
Kaffe, traktet, filter	☐	☐	☐	☐	☐	☐	☐	☐	☐
Kaffe, pulver (instant)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Kaffe, koffeinfri	☐	☐	☐	☐	☐	☐	☐	☐	☐
Te	☐	☐	☐	☐	☐	☐	☐	☐	☐
Nypete, urtete	☐	☐	☐	☐	☐	☐	☐	☐	☐

	Antall teskjeer eller biter pr. kopp					
	0	1/2	1	2	3	4+
Sukker til kaffe	☐	☐	☐	☐	☐	☐
Sukker til te	☐	☐	☐	☐	☐	☐
Kunstig søtstoff til kaffe eller te	☐	☐	☐	☐	☐	☐
Fløte til kaffe	☐	☐	☐	☐	☐	☐

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9. ANDRE DRIKKER?

Svar enten pr. måned **eller** pr. uke. < 1 betyr sjeldnere enn 1 gang.
Merk at porsjonsenhetene er forskjellige. 1/3 liter tilsvarer en halvflaske øl
og 2/3 liter tilsvarer en helflaske.

	Gang pr. måned					Gang pr. uke						Mengde pr. gang					
	0	<1	1	2	3	1	2-3	4-5	6-7	8+							
Vann	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(glass)	1/2 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5+ ☐
Appelsinjuice	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(glass)	1/2 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5+ ☐
Annen juice, most, nektar	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(glass)	1/2 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5+ ☐
Saft, solbærsirup m. sukker	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(glass)	1/2 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5+ ☐
Saft, kunstig søtet	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(glass)	1/2 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5+ ☐
Brus, Cola, Solo o.l., med sukker	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(liter)	1/4 ☐	1/3 ☐	1/2 ☐	2/3 ☐	1 ☐	11/2+ ☐
Brus, Cola, Solo o.l., kunstig søtet	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(liter)	1/4 ☐	1/3 ☐	1/2 ☐	2/3 ☐	1 ☐	11/2+ ☐
Farris, Selters, Soda o.l.	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(liter)	1/4 ☐	1/3 ☐	1/2 ☐	2/3 ☐	1 ☐	11/2+ ☐
Alkoholfritt øl, vørterøl, lettøl	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(liter)	1/4 ☐	1/3 ☐	1/2 ☐	2/3 ☐	1 ☐	11/2+ ☐
Pilsnerøl	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(liter)	1/4 ☐	1/3 ☐	1/2 ☐	2/3 ☐	1 ☐	11/2+ ☐
Vin	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(glass)	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6+ ☐
Brennevin, likør	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(1 dram = 4 cl)	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6+ ☐

10. MIDDAGSRETTER

Vi spør både om middagsmåltidene og det du spiser til andre måltider. Tell til slutt
sammen antall retter du har merket for og se om summen virker sannsynlig.
En "dl" tilsvarer omtrent mengden i en suppeøse. Med "ss" menes en spiseskje.

	Gang pr. måned										Mengde pr. gang				
	0	<1	1	2	3	4	5-6	7-8	9+							
Kjøttpølse, medisterpølse	☐	☐	☐	☐	☐	☐	☐	☐	☐	(kjøttpølse)	1/2 ☐	2/3 ☐	1 ☐	11/2 ☐	2+ ☐
Hamburger, karbonader o.l.	☐	☐	☐	☐	☐	☐	☐	☐	☐	(stk)	1 ☐	2 ☐	3 ☐	4 ☐	5+ ☐
Grill- og wienerpølse	☐	☐	☐	☐	☐	☐	☐	☐	☐	(pølse)	1 ☐	2 ☐	3 ☐	4 ☐	5+ ☐
Hamburger-, pølsebrød, lomper	☐	☐	☐	☐	☐	☐	☐	☐	☐	(stk)	1 ☐	2 ☐	3 ☐	4 ☐	5+ ☐
Kjøttkaker, medisterkaker, kjøttpudding	☐	☐	☐	☐	☐	☐	☐	☐	☐	(stk)	1 ☐	2 ☐	3 ☐	4 ☐	5+ ☐
Kjøttdeigretter (saus eller gryte med kjøttdeig, lasagne o.l.)	☐	☐	☐	☐	☐	☐	☐	☐	☐	(dl)	1 ☐	2 ☐	3 ☐	4 ☐	5+ ☐
Taco (med kjøtt og salat)	☐	☐	☐	☐	☐	☐	☐	☐	☐	(stk)	1 ☐	2 ☐	3 ☐	4 ☐	5+ ☐
Pastaretter	☐	☐	☐	☐	☐	☐	☐	☐	☐	(dl)	1 ☐	2 ☐	3 ☐	4 ☐	5+ ☐

Gang pr. måned — Mengde pr. gang

Matvare	Gang pr. måned (0, <1, 1, 2, 3, 4, 5-6, 7-8, 9+)	Enhet	Mengde pr. gang
Pizza (500-600 g)	☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐	(pizza)	1/8 1/4 1/2 3/4 1+ — ☐ ☐ ☐ ☐ ☐
Biff (alle typer kjøtt)	☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐	(stk)	1/2 1 1 1/2 2 2 1/2+ — ☐ ☐ ☐ ☐ ☐
Koteletter (lam, okse, svin)	☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐	(stk)	1/2 1 1 1/2 2 2 1/2+ — ☐ ☐ ☐ ☐ ☐
Stek (lam, okse, svin)	☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐	(skive)	1-2 3-4 5-6 7-8 9+ — ☐ ☐ ☐ ☐ ☐
Stek (elg, hjort, reinsdyr o.l.)	☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐	(skive)	1-2 3-4 5-6 7-8 9+ — ☐ ☐ ☐ ☐ ☐
Gryterett med helt kjøtt, frikassé, fårikål o.l.	☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐	(dl)	1-2 3-4 5-6 7-8 9+ — ☐ ☐ ☐ ☐ ☐
Lapskaus, suppelapskaus, betasuppe	☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐	(dl)	1-2 3-4 5-6 7-8 9+ — ☐ ☐ ☐ ☐ ☐
Bacon, stekt flesk	☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐	(skive)	1-2 3-4 5-6 7-8 9+ — ☐ ☐ ☐ ☐ ☐
Kylling, høne	☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐	(stk)	1/4 1/3 1/2 3/4 1+ — ☐ ☐ ☐ ☐ ☐
Leverretter	☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐	(skive)	1-2 3-4 5-6 7-8 9+ — ☐ ☐ ☐ ☐ ☐
Fiskekaker, fiskepudding, fiskeboller	☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐	(kake)	1 2 3 4 5+ — ☐ ☐ ☐ ☐ ☐
Fiskepinner	☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐	(stk)	1-2 3-4 5-6 7-9 10+ — ☐ ☐ ☐ ☐ ☐
Torsk, sei, hyse (kokt)	☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐	(stk)	1 2 3 4 5+ — ☐ ☐ ☐ ☐ ☐
Torsk, sei, hyse (stekt, panert)	☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐	(stk)	1 2 3 4 5+ — ☐ ☐ ☐ ☐ ☐
Sild (fersk, speket, røkt)	☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐	(filet)	1 2 3 4 5+ — ☐ ☐ ☐ ☐ ☐
Makrell (fersk, røkt)	☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐	(filet)	1/2 1 1 1/2 2 3+ — ☐ ☐ ☐ ☐ ☐
Laks, ørret (sjø, oppdrett)	☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐	(skive)	1 2 3 4 5+ — ☐ ☐ ☐ ☐ ☐
Fiskegryte, -grateng, suppe med fisk	☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐	(dl)	1-2 3-4 5-6 7-8 9+ — ☐ ☐ ☐ ☐ ☐
Reker, krabbe	☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐	(dl, renset)	1 2 3 4 5+ — ☐ ☐ ☐ ☐ ☐
Risgrøt, annen melkegrøt	☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐	(dl)	1-2 3-4 5-6 7-8 9+ — ☐ ☐ ☐ ☐ ☐
Pannekaker	☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐	(stk)	1-2 3-4 5-6 7-8 9+ — ☐ ☐ ☐ ☐ ☐
Suppe (tomat, blomkål, ertesuppe o.l.)	☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐	(dl)	1-2 3-4 5-6 7-8 9+ — ☐ ☐ ☐ ☐ ☐
Vegetarrett, vegetarpizza grønnsakgrateng, -pai	☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐	(bit/dl)	1-2 3-4 5-6 7-8 9+ — ☐ ☐ ☐ ☐ ☐
Brun/hvit saus	☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐	(dl)	1/2 1 1 1/2 2 2 1/2+ — ☐ ☐ ☐ ☐ ☐
Smeltet margarin, smør til fisk	☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐	(ss)	1-2 3-4 5-6 7-8 9+ — ☐ ☐ ☐ ☐ ☐
Bearnaisesaus o.l.	☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐	(ss)	1 2 3 4 5+ — ☐ ☐ ☐ ☐ ☐
Majones, remulade	☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐	(ss)	1 2 3 4 5+ — ☐ ☐ ☐ ☐ ☐
Ketchup	☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐	(ss)	1 2 3 4 5+ — ☐ ☐ ☐ ☐ ☐

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Svar enten pr. måned **eller** pr. uke. <1 betyr sjeldnere enn 1 gang.
Disse spørsmålene dreier seg først og fremst om tilbehør til middagsretter, men spiser du for eksempel en rå gulrot eller salat til lunsj, skal det tas med her.

	Gang pr. måned					Gang pr. uke						Mengde pr. gang				
	0	<1	1	2	3	1	2-3	4-5	6-7	8+						
Poteter, kokte	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(stk)	1 ☐	2 ☐	3 ☐	4 ☐	5+ ☐
Pommes frites, stekte poteter	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(dl)	1 ☐	2 ☐	3 ☐	4 ☐	5+ ☐
Potetmos, -stuing, gratinerte poteter	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(dl)	1 ☐	2 ☐	3 ☐	4 ☐	5+ ☐
Ris	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(dl)	1-2 ☐	3-4 ☐	5-6 ☐	7-8 ☐	9+ ☐
Spaghetti, makaroni, pasta	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(dl)	1-2 ☐	3-4 ☐	5-6 ☐	7-8 ☐	9+ ☐
Gulrot	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(stk)	1/2 ☐	1 ☐	1 1/2 ☐	2 ☐	3+ ☐
Hodekål	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(skalk)	1 ☐	2 ☐	3 ☐	4 ☐	5+ ☐
Kålrot	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(skive)	1 ☐	2 ☐	3 ☐	4 ☐	5+ ☐
Blomkål	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(bukett)	1-2 ☐	3-4 ☐	5-6 ☐	7-8 ☐	9+ ☐
Brokkoli	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(bukett)	1-2 ☐	3-4 ☐	5-6 ☐	7-8 ☐	9+ ☐
Rosenkål	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(stk)	1-2 ☐	3-4 ☐	5-6 ☐	7-8 ☐	9+ ☐
Grønnkål	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(dl)	1 ☐	2 ☐	3 ☐	4 ☐	5+ ☐
Løk	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(ss)	1 ☐	2 ☐	3 ☐	4 ☐	5+ ☐
Spinat, andre bladgrønns.	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(dl)	1 ☐	2 ☐	3 ☐	4 ☐	5+ ☐
Sopp	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(stk)	1-2 ☐	3-4 ☐	5-6 ☐	7-8 ☐	9+ ☐
Avocado	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(stk)	1/4 ☐	1/2 ☐	3/4 ☐	1 ☐	1 1/4 + ☐
Paprika	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(strimmel)	1 ☐	2 ☐	3 ☐	4 ☐	5+ ☐
Tomat	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(stk)	1/2 ☐	1 ☐	1 1/2 ☐	2 ☐	3+ ☐
Tomatbønner, bønner/linser	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(dl)	1 ☐	2 ☐	3 ☐	4 ☐	5+ ☐
Mais	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(ss)	1-2 ☐	3-4 ☐	5-6 ☐	7-8 ☐	9+ ☐
Erter, frosne grønnsak-blandinger	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(dl)	1 ☐	2 ☐	3 ☐	4 ☐	5+ ☐
Salatblandinger	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(dl)	1 ☐	2 ☐	3 ☐	4 ☐	5+ ☐
Dressing	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(ss)	1/2 ☐	1 ☐	2 ☐	3 ☐	4+ ☐
Rømme	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(ss)	1/2 ☐	1 ☐	2 ☐	3 ☐	4+ ☐

Hvor mange ganger om dagen spiser du vanligvis grønnsaker utenom grønnsakene du spiser til middag?

0	1	2	3	4	5+
☐	☐	☐	☐	☐	☐

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12. TYPE FETT TIL MATLAGING

Smør/margarin

☐ Smør (meierismør)

☐ Bremykt

☐ Melange, Per

☐ Soft-, soyamargarin (pakke, beger)

☐ Solsikke

☐ Oliven

☐ Annen margarin

Oljer

☐ Olivenolje

☐ Soyaolje

☐ Maisolje

☐ Solsikkeolje

☐ Valnøttolje

☐ Andre oljer

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13. FRUKT

Svar enten pr. måned **eller** pr. uke. < 1 betyr sjeldnere enn 1 gang.

	Gang pr. måned					Gang pr. uke						Mengde pr. gang			
	0	<1	1	2	3	1	2-3	4-5	6-7	8+		1/2	1	2	3+
Eple	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(stk)	☐	☐	☐	☐
Appelsin, mandarin, grapefrukt	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(stk)	☐	☐	☐	☐
Banan	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(stk)	☐	☐	☐	☐
Druer	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(klase)	☐	☐	☐	☐
Eksotisk frukt (kiwi, mango)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(stk)	☐	☐	☐	☐
Annen frukt (fersken, pære m.v.)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(stk)	☐	☐	☐	☐
Jordbær, bringebær (friske, frosne)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(dl)	☐	☐	☐	☐
Blåbær	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(dl)	☐	☐	☐	☐
Multer	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(dl)	☐	☐	☐	☐

Hvor mange frukter spiser du vanligvis pr. dag?	0	1	2	3	4	5	6	7	8	9+
	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

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14. DESSERT, KAKER, GODTERI

Svar enten pr. måned **eller** pr. uke. < 1 betyr sjeldnere enn 1 gang.

	Gang pr. måned					Gang pr. uke						Mengde pr. gang			
	0	<1	1	2	3	1	2-3	4-5	6-7	8+					
Hermetisk frukt, fruktgrøt	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(dl)	1/2 ☐	1 ☐	2 ☐	3+ ☐
Puddinger (sjokolade, karamell o.l.)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(dl)	1 ☐	2 ☐	3 ☐	4+ ☐
Is (1 dl = 1 pinne = 1 kremmerhus)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(dl)	1 ☐	2 ☐	3 ☐	4+ ☐
Boller, julekake, kringle	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(stk)	1 ☐	2 ☐	3 ☐	4+ ☐
Skolebrød, skillingsbolle	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(stk)	1 ☐	2 ☐	3 ☐	4+ ☐
Wienerbrød, -kringle o.l.	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(stk)	1 ☐	2 ☐	3 ☐	4+ ☐
Smultring, formkake	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(stk)	1 ☐	2 ☐	3 ☐	4+ ☐
Vafler	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(plate)	1/2 ☐	1 ☐	2 ☐	3+ ☐
Sjokoladekake, bløtkake, annen fylt kake	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(stk)	1/2 ☐	1 ☐	2 ☐	3+ ☐
Søt kjeks, kakekjeks (Cookies, Bixit, Hob Nobs)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(stk)	1-2 ☐	3-4 ☐	5-6 ☐	7+ ☐
Sjokolade (60 g)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(plate)	1/2 ☐	1 ☐	2 ☐	3+ ☐
Drops, lakris, seigmenn o.l.	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(stk)	1-2 ☐	3-4 ☐	5-6 ☐	7+ ☐
Smågodt (1 hg = 100g)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(hg)	1/2 ☐	3/4 ☐	1 ☐	1 1/2+ ☐
Potetgull (1 pose 100g = 7 dl)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(dl)	1-2 ☐	3-4 ☐	5-6 ☐	7+ ☐
Annen snacks (skruer, crisp, saltstenger, lettsnacks o.l.)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(dl)	1-2 ☐	3-4 ☐	5-6 ☐	7+ ☐
Peanøtter, andre nøtter (1 pose 100g = 4 never)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	(neve)	1 ☐	2 ☐	3 ☐	4+ ☐

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15. KOSTTILSKUDD (bs = barneskje, ts = teskje)

	Hele året	Bare vinter-halvåret	**Gang pr. uke**						**Mengde pr. gang**			
			0	<1	1	2-3	4-5	6-7				
									1 ts	1 bs	1 ss	
Tran	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	
									1	2+		
Trankapsler	☐	☐	☐	☐	☐	☐	☐	☐	kapsler ☐	☐		
									1-2	3-4	5-6	7+
Fiskeoljekapsler	☐	☐	☐	☐	☐	☐	☐	☐	kapsler ☐	☐	☐	☐

Multipreparater

	Hele året	Bare vinter-halvåret	0	<1	1	2-3	4-5	6-7		1	2	3	4+
Sanasol	☐	☐	☐	☐	☐	☐	☐	☐	bs	☐	☐	☐	☐
Biovit	☐	☐	☐	☐	☐	☐	☐	☐	bs	☐	☐	☐	☐
Vitaplex	☐	☐	☐	☐	☐	☐	☐	☐	tablett	☐	☐	☐	☐
Kostpluss	☐	☐	☐	☐	☐	☐	☐	☐	tablett	☐	☐	☐	☐
Vitamineral	☐	☐	☐	☐	☐	☐	☐	☐	tablett	☐	☐	☐	☐
Annet	☐	☐	☐	☐	☐	☐	☐	☐	tablett	☐	☐	☐	☐

Hvis annet, hvilket? ....

Jernpreparater

	Hele året	Bare vinter-halvåret	0	<1	1	2-3	4-5	6-7		1	2	3	4+
Ferro C	☐	☐	☐	☐	☐	☐	☐	☐	tablett	☐	☐	☐	☐
Hemofer	☐	☐	☐	☐	☐	☐	☐	☐	tablett	☐	☐	☐	☐
Duroferon Duretter	☐	☐	☐	☐	☐	☐	☐	☐	tablett	☐	☐	☐	☐
Annet	☐	☐	☐	☐	☐	☐	☐	☐	tablett	☐	☐	☐	☐

Hvis annet, hvilket? ....

	Hele året	Bare vinter-halvåret	0	<1	1	2-3	4-5	6-7		1	2	3	4+
B-vitaminer	☐	☐	☐	☐	☐	☐	☐	☐	tablett	☐	☐	☐	☐
C-vitamin	☐	☐	☐	☐	☐	☐	☐	☐	tablett	☐	☐	☐	☐
D-vitamin	☐	☐	☐	☐	☐	☐	☐	☐	tablett	☐	☐	☐	☐
E-vitamin	☐	☐	☐	☐	☐	☐	☐	☐	tablett	☐	☐	☐	☐
Folat (folsyre)	☐	☐	☐	☐	☐	☐	☐	☐	tablett	☐	☐	☐	☐

	Hele året	Bare vinter-halvåret	0	<1	1	2-3	4-5	6-7		1	2	3	4+
Kalktabletter	☐	☐	☐	☐	☐	☐	☐	☐	tablett	☐	☐	☐	☐
Fluortabletter	☐	☐	☐	☐	☐	☐	☐	☐	tablett	☐	☐	☐	☐
Annet	☐	☐	☐	☐	☐	☐	☐	☐	tablett	☐	☐	☐	☐

Hvis annet, hvilket? ....

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16. NÅR SPISER DU PÅ HVERDAGER?

HOVEDMÅLTIDER som frokost, formiddagsmat, middag, kvelds.

Omtrent klokken

6	8	10	12	14	16	18	20	22	24	2	4
☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐

MELLOMMÅLTIDER som kaffe, frukt, godteri, snacks m.v.

Omtrent klokken

6	8	10	12	14	16	18	20	22	24	2	4
☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐

17. MENER DU SVARENE I SPØRRESKJEMAET GIR ET BRUKBART BILDE AV KOSTHOLDET DITT?

Ja ☐ Nei ☐

Er det matvarer/produkter du regelmessig bruker, og som ikke er nevnt i skjemaet?

....

....

18. ER DU FORNØYD MED KROPPSVEKTEN DIN SLIK DEN ER NÅ?

☐ Ja

☐ Nei, jeg ønsker å slanke meg

☐ Nei, jeg ønsker å legge på meg

19. KJØNN

Mann ☐ Kvinne ☐

Vennligst se etter at du har svart på alle spørsmål.

Takk for innsatsen!

VI

CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	1
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	2
	2b	Specific objectives or hypotheses	2
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	2
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	3
Participants	4a	Eligibility criteria for participants	2
	4b	Settings and locations where the data were collected	2
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	2
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	Primary: 3, Secondary:4
	6b	Any changes to trial outcomes after the trial commenced, with reasons	3
Sample size	7a	How sample size was determined	4
	7b	When applicable, explanation of any interim analyses and stopping guidelines	2
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	2
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	2
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	2
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	-
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those	2

Section/Topic	Item No	Checklist item	Reported on page No
		assessing outcomes) and how	
	11b	If relevant, description of the similarity of interventions	NA
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	4
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	4
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	3
	13b	For each group, losses and exclusions after randomisation, together with reasons	3
Recruitment	14a	Dates defining the periods of recruitment and follow-up	2
	14b	Why the trial ended or was stopped	NA
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	5
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	3, 5, 6
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	6
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	Suppl table 1
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	Suppl
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	Suppl table 3
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	8
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	8
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	6-8
Other information			
Registration	23	Registration number and name of trial registry	1
Protocol	24	Where the full trial protocol can be accessed, if available	
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.
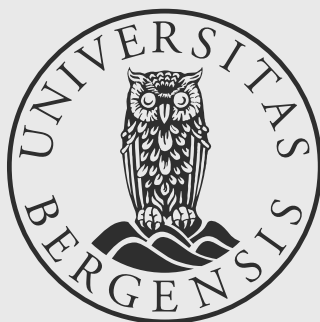
VII

CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	434
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	434
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	434-435
	2b	Specific objectives or hypotheses	435
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	435
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	435
Participants	4a	Eligibility criteria for participants	435
	4b	Settings and locations where the data were collected	435
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	435
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	435-436
	6b	Any changes to trial outcomes after the trial commenced, with reasons	-
Sample size	7a	How sample size was determined	436
	7b	When applicable, explanation of any interim analyses and stopping guidelines	435 and 438
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	435
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	435
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	435
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	435
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those	435

Section/Topic	Item No	Checklist item	Reported on page No
		assessing outcomes) and how	
	11b	If relevant, description of the similarity of interventions	436
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	436
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	-
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	436 (flow chart)
	13b	For each group, losses and exclusions after randomisation, together with reasons	436
Recruitment	14a	Dates defining the periods of recruitment and follow-up	Only recruitment, 435
	14b	Why the trial ended or was stopped	NA
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	Table 1, 437
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	438
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	Not CI, 438
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	NA
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	438
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	438
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	439
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	439
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	438-439
Other information			
Registration	23	Registration number and name of trial registry	435
Protocol	24	Where the full trial protocol can be accessed, if available	-
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	Role of funder not declared, 440

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